SCUOLA di DOTTORATO

Dottorato in Architettura
Dottorato in Diritto ed Economia
Dottorato in Ingegneria Civile, Ambientale e Industriale
Dottorato in Ingegneria dell'Informazione
Dottorato in Scienze Agrarie, Alimentari e Forestali

Direttore della Scuola di Dottorato
prof. Felice Arena

Collegio dei docenti
Dottorato di Ricerca in Scienze Agrarie, Alimentari e Forestali
XXXVIII ciclo

Abdelfattah Ahmed
Abenavoli Maria Rosa
Agosteo Giovanni
Barreca Francesco
Bernardi Bruno
Bombino Giuseppe
Campolo Orlando
De Luca Anna Irene
Di Fazio Salvatore
García-Ruíz Roberto
Giuffrè Angelo Maria
Gulisano Giovanni
Li Destri Nicosia Maria Giulia
Lombardi Fabio
Lucas-Borja Manuel Esteban
Lupini Antonio
Marcianò Claudio
Marziliano Pasquale
Modica Giuseppe
Monti Michele
Muscolo Adele Maria
Nicolosi Agata
Palmeri Vincenzo
Piscopo Amalia Rosa Maria
Poiana Marco
Porto Paolo
Proto Andrea Rosario
Schena Leonardo
Sicari Vincenzo
Sorgonà Agostino
Spampinato Giovanni
Strano Alfio
Sunseri Francesco
Tassone Valentina C.
Zema Demetrio Antonio
Zimbalatti Giuseppe

Dottorato di Ricerca
Scienze Agrarie Alimentari e Forestali

SCUOLA DI DOTTORATO
Università Mediterranea di Reggio Calabria

DIPARTIMENTO
Di Agraria

DOTTORATO DI RICERCA
Scienze Agrarie, Alimentari e Forestali

S.S.D. AGR-06/B
XXXVII CICLO

Innovative Strategies to Protect Natural Resources and Reduce the Environmental Impact of Agriculture

Solutions for Soil Quality Assessment and Protection

Dottoranda
Mariateresa Oliva
[redacted]

Tutor prof.
Adele Maria Muscolo
[redacted]

DIPARTIMENTO DI AGRARIA
UNIVERSITÀ DEGLI STUDI MEDITERRANEA DI REGGIO CALABRIA

Coordinatore del Dottorato
prof. Leonardo Schena
[redacted]

Indice

Abstract/Sommario 1

Chapter 1 – Introduction 3

1.1 Soil as a Strategic Resource 4

1.2 Soil Quality and Soil Health: Concepts, Criteria, and Assessment Approaches 5

1.3 Sustainable Management and Circular Fertilization Strategies 7

1.4 Soil Biodiversity and Ecosystem Functions 9

1.5 Integration of Innovative Assessment Methods 12

Reference 14

PhD Thesis Objectives 21

Reference 22

Results Organization 24

Additional papers 25

Chapter 2 - Humic Substances: Bridging Ecology and Agriculture for a Greener Future 27

Chapter 3 - Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli 61

Chapter 4 - Circular Fertilization Strategy Using Sulphur with Orange Waste Enhances Soil Health and Broccoli Nutritional and Nutraceutical Quality in Mediterranean Systems 83

Chapter 5 - A Multi-Indicator Approach to Soil Quality in Agroecological Systems Based on the Use of Organic Waste within a Circular Economy Model 107

Chapter 6 — General Discussion and Conclusions 133

6.1 Synthesis and Comparison of Results 133

6.2 Scientific Contributions of the Thesis 135

6.2.1 Methodological Innovations 135

6.2.2 Multifactorial Indicators of Soil Quality 135

6.2.3 Impacts on Mediterranean Agroecology 136

6.3 Practical and Policy Implications for Organic and Circular Agriculture 137

6.4 Future Research Perspectives 138

Appendix- Additional papers 139

Sustainable Soil Management: The Dynamic Impact of Combined Use of Crop Rotation and Fertilizers from Agri-Food and Sulfur Hydrocarbon Refining Processes Wastes 139

Impacts of Conventional and Agri-Food Waste-Derived Fertilizers on Durum Wheat Yield, Grain Quality, and Soil Health: A Two-Year Field Study in Greece and Southern Italy 165

Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli 181

Humic Substances from Waste-Based Fertilizers for Improved Soil Fertility 203

Exploring the Potential and Obstacles of Agro-Industrial Waste-Based Fertilizer 224

Abstract/Sommario

Soil degradation in Mediterranean agroecosystems, characterised by low organic matter, high hydroclimatic variability, and increasing anthropogenic pressure, compromises the processes that underpin soil fertility, nutrient availability, and ecosystem resilience. In this context, circular fertilizers derived from agro-industrial residues represent an effective strategy to restore soil functionality and reduce dependence on mineral inputs. This thesis provides a comparative evaluation of farmyard manure, mineral NPK, newly developed composts and vermicomposts (produced from sawdust and source-separated organic waste), and the innovative RecOrgFert PLUS fertilizer (elemental sulphur combined with citrus waste) on soil quality and crop responses in Mediterranean horticultural systems. An integrated framework was applied, combining chemical, biological, biochemical, microbiological, physical, and faunistic indicators, including pH, cation exchange capacity, organic carbon, organic matter, C/N ratio, soil moisture, microbial biomass, enzymatic activities, and the QBS-ar index.

Multivariate analyses enabled a comprehensive interpretation of the interactions among these indicators, highlighting key processes associated with carbon dynamics, nutrient availability, and soil biodiversity. Traditional fertilizers such as manure and NPK generated limited and short-term effects, without promoting substantial improvements in soil functioning. In contrast, RecOrgFert PLUS elicited the strongest responses, enhancing biological and biochemical soil parameters and improving broccoli nutritional quality.

Compost and vermicompost further demonstrated a marked capacity to enhance soil quality by increasing organic carbon, cation exchange capacity, microbial activity, and enzymatic processes. Lignocellulosic matrices favoured the accumulation of stable organic matter, whereas more biodegradable mixtures stimulated rapid microbial activation. The QBS-ar index confirmed the superior effectiveness of organic matrices in improving soil biological quality, highlighting significant increases in the diversity and abundance of edaphic fauna that were not observed in soils treated with mineral NPK fertilizers. The results demonstrate that the regeneration of Mediterranean soils requires a synergistic activation of the chemical, biological, enzymatic, and faunal components of the edaphic system. In this context, circular fertilizers emerge as effective tools for enhancing soil functionality, improving the quality of agricultural production, and promoting the sustainability of Mediterranean cropping systems.

Keywords: Soil enzymatic activity; Edaphic biodiversity (QBS-ar); Microbial biodiversity and biomass; Bio-based fertilizers; Soil quality; Compost and vermicompost.

Il degrado del suolo negli agroecosistemi mediterranei, caratterizzati da ridotto contenuto di sostanza organica, elevata variabilità idro-climatica e crescente pressione antropica, compromette i processi che sostengono la fertilità del suolo, la disponibilità dei nutrienti e la resilienza ecosistemica. In questo contesto, i fertilizzanti circolari derivati da residui agro-industriali rappresentano una strategia efficace per ripristinare la funzionalità edafica e ridurre la dipendenza dagli input minerali. Questa tesi fornisce una valutazione comparativa degli effetti di stallatico, fertilizzante minerale NPK, nuovi compost e vermicompost (prodotti da segatura e frazione organica umida) e del fertilizzante innovativo RecOrgFert PLUS (zolfo elementare combinato con pastazzo di agrumi) sulla qualità del suolo e sulle risposte colturali in sistemi orticoli mediterranei. È stato adottato un framework integrato che combina indicatori chimici, biologici, biochimici, microbiologici, fisici e faunistici, tra cui pH, capacità di scambio cationico, carbonio organico, sostanza organica, rapporto C/N, contenuto idrico, biomassa microbica, attività enzimatiche e indice QBS-ar. Le analisi multivariate hanno consentito un'interpretazione completa delle interazioni tra tali indicatori, evidenziando i processi chiave associati alla dinamica del carbonio, alla disponibilità dei nutrienti e alla biodiversità del suolo. I fertilizzanti tradizionali, quali stallatico e NPK, hanno prodotto effetti limitati e di breve durata, senza promuovere miglioramenti sostanziali della funzionalità del suolo. Al contrario, RecOrgFert PLUS ha suscitato le risposte più marcate, incrementando i parametri biologici e biochimici del suolo e migliorando la qualità nutrizionale dei broccoli. Compost e vermicompost hanno ulteriormente dimostrato un'elevata capacità di miglioramento della qualità del suolo, aumentando il carbonio organico, la capacità di scambio cationico, l'attività microbica e i processi enzimatici. Le matrici lignocellulosiche hanno favorito l'accumulo di sostanza organica stabile, mentre quelle più biodegradabili hanno stimolato una rapida attivazione microbica. L'indice QBS-ar ha confermato la superiore efficacia delle matrici organiche nel migliorare la qualità biologica del suolo, evidenziando incrementi significativi nella diversità e nell'abbondanza della fauna edafica, non riscontrati nei suoli trattati con fertilizzanti minerali NPK. I risultati dimostrano che la rigenerazione dei suoli mediterranei richiede un'attivazione sinergica delle componenti chimiche, biologiche, enzimatiche e faunistiche del sistema edafico. In tale contesto, i fertilizzanti circolari si configurano come strumenti efficaci per il potenziamento della funzionalità del suolo, il miglioramento della qualità delle produzioni agricole e la promozione della sostenibilità dei sistemi colturali mediterranei.

Parole chiavi: Attività enzimatica del suolo; Biodiversità edafica (QBS-ar); Biodiversità e biomassa microbica; Fertilizzanti bio-based; qualità del suolo; Compost e vermicompost.

Chapter 1 – Introduction

Soil represents an essential component of terrestrial ecosystems and a key determinant of agricultural productivity and environmental stability. According to the FAO (2023), soil health is closely linked to overall ecosystem quality and, indirectly, to human well-being, as soil degradation undermines water and air quality as well as food security. This fragility is exacerbated by current climatic instability, characterised by increasing drought, salinisation, erosion, and extreme weather events (FAO et al., 2020). Although agricultural intensification has enhanced yields, it has also led to a decline in soil organic matter, a loss of microbial biodiversity, and the deterioration of soil structure and functionality. These effects are particularly pronounced in Mediterranean systems, which are intrinsically vulnerable to thermal and hydric stress (Carswell et al., 2025; Intergovernmental Panel on Climate Change (IPCC), 2022). The resulting loss of pedological functionality reduces the resilience of agroecosystems and their capacity to provide essential ecosystem services. In this context, the paradigm of Sustainable Intensification (SI) aims to increase resource-use efficiency and reduce environmental impacts while maintaining agricultural productivity (Denning et al., 2025). However, soil responses to management practices are strongly influenced by pedoclimatic conditions and biological dynamics, making it necessary to employ analytical tools capable of capturing soil complexity and functional heterogeneity. Recent European policies (including the Green Deal and the EU Soil Strategy for 2030) recognise soil as a strategic resource for ecological transition, promoting regenerative practices, the restoration of soil organic matter, and the valorisation of organic residues within a circular economy framework (Montanarella & Panagos, 2021). This shift requires more advanced and harmonised monitoring approaches capable of integrating physical, chemical, biological, and biochemical indicators to effectively characterise soil functioning and support evidence-based soil governance (Panagos et al., 2024). Responding to these needs, the present research adopts a multi-indicator assessment framework to evaluate the effects of innovative and bio-based fertilizers on soil quality in Mediterranean agroecosystems. The main aim is to provide scientifically robust tools to support the transition towards more resilient, regenerative, and climate-sustainable agricultural systems.

1.1 Soil as a Strategic Resource

Soil is a strategic and scarcely renewable natural resource, underpinning the functioning of terrestrial ecosystems and the productivity of agricultural systems. Its multidimensional nature arises from the integration of physical, chemical, and biological properties, which collectively determine the soil's capacity to support key processes such as primary production, biogeochemical cycling, biodiversity conservation, and ecological stability (Lehmann et al., 2020). From this perspective, soil must be regarded as a living system, characterised by complex metabolic dynamics and strong interactions among microbial communities, organic matter, and the mineral matrix.

The role of soil as natural capital is evident in the wide range of ecosystem services it provides, including regulating and supporting functions driven by physical, chemical and biological components, as well as cultural ecosystem services shaped by human–soil interactions and landscape management (Gould & Satterfield, 2025). This functional integration enables soil to support provisioning, regulating, supporting, and cultural ecosystem services, as consistently highlighted by global ecosystem assessment frameworks, including those developed under the IPBES (2019).

A particularly relevant function is the role of soil in the global carbon cycle. With an estimated stock of 2,300–2,400 Gt C, soils represent the largest terrestrial reservoir of organic carbon (IPCC, 2022). Carbon stabilisation depends on microbial decomposition, humification, and the formation of organo-mineral complexes, processes strongly influenced by organic matter quality and soil mineralogy (Lehmann & Kleber, 2015). Agronomic practices that increase organic matter inputs, reduce soil disturbance, and maintain vegetation cover enhance the soil's capacity to accumulate stable carbon, while concurrently improving fertility, structure, and biogeochemical resilience (Sharma et al., 2024; Smith et al., 2020).

Beyond carbon sequestration, soils play a key role in regulating greenhouse gas emissions through microbially mediated processes such as nitrification, denitrification, methanogenesis, and methane oxidation. These processes are strongly influenced by nutrient availability, soil moisture, and microbial activity, and are highly sensitive to land management practices (Bhattacharyya, 2024). The soil's contribution to climate-change mitigation is therefore closely linked to its biological integrity and physico-chemical

condition.

Soil formation occurs over extremely long temporal scales, requiring centuries to millennia to develop only a few centimetres of fertile soil. Consequently, soil is effectively a non-renewable resource on a human timescale, whose degradation directly threatens ecosystem functioning, agricultural productivity, and environmental security, particularly in vulnerable regions such as the Mediterranean (IPBES, 2019; FAO et al., 2020; Montanarella & Panagos, 2021). This limited renewability, combined with increasing pressure from intensive agricultural use and climatic stresses, underscores the need for management strategies that preserve soil multifunctionality and enhance its capacity to support resilient agroecosystems. Within the European context, soil is increasingly recognised as natural capital and a strategic resource whose protection and regeneration are essential to achieving environmental and climate objectives (Montanarella & Panagos, 2021).

Soil thus constitutes an essential resource for ecosystem functioning and for the stability of agro-environmental systems. Its protection requires an approach grounded in an integrated understanding of the physical, chemical, and biological processes that govern it, as well as in the valorisation of the ecosystem services it provides, with growing attention to the conservation of soil biodiversity and to the soil's role in climate mitigation.

1.2 Soil Quality and Soil Health: Concepts, Criteria, and Assessment Approaches

Soil quality and soil health represent two foundational concepts for evaluating the functioning of agroecosystems and guiding agricultural management towards more sustainable production systems. Both describe the soil's capacity to support essential ecological functions (productive, biogeochemical, and biological) while maintaining stability and resilience over time in the face of climatic and anthropogenic pressures (FAO, 2021; Lehmann et al., 2020).

Soil quality is defined as the capacity of soil to sustain crop productivity, support edaphic biodiversity, and maintain the provision of essential ecosystem services, including nutrient cycling, water regulation, and structural stability within agroecosystems (Lehmann et al., 2020; Janzen et al., 2021). It integrates physical, chemical, and biological parameters that collectively describe the functional status of soils under both natural and managed conditions, providing a robust basis for interpreting soil functioning in terms of ecosystem

services and multifunctionality (Abshiba et al., 2025; Ahmad et al., 2024; El Behairy et al., 2024; Hu et al., 2024).

Soil health, while overlapping substantially with soil quality, places stronger emphasis on the biological vitality of the soil system and its capacity to self-regenerate, withstand stress, and maintain efficient ecological cycles (Bünemann et al., 2018; Lehmann et al., 2020). Soil health, while overlapping substantially with soil quality, places stronger emphasis on the biological vitality of the soil system, its functional diversity, and its capacity to self-regenerate, withstand disturbances, and sustain efficient biogeochemical cycles, which are central to ecosystem resilience and sustainability (Bünemann et al., 2018; Bhaduri et al., 2022).

The operational distinction between soil quality and soil health is not merely terminological: soil quality reflects the suitability of soil to support productive and agroecosystem functions, whereas soil health highlights the biological component and the stability of processes that sustain soil as a living ecosystem. Employing both concepts jointly allows the development of integrated agronomic strategies that enhance productivity in a regenerative manner while preserving ecological functionality.

Assessing soil quality and soil health requires the combined use of physical, chemical, biological, and biochemical indicators, each providing complementary insights into soil system dynamics. Physical indicators describe soil structure, porosity, and water dynamics: bulk density, aggregate stability, hydraulic conductivity, and water retention curves are critical parameters for understanding infiltration, aeration, and water availability in Mediterranean environments (Wang & Meng, 2024; Abshiba et al., 2025). Despite their importance, these indicators exhibit strong spatial heterogeneity and respond rapidly to management.

Chemical indicators reflect potential soil fertility and nutrient availability. Parameters such as pH, electrical conductivity (EC), cation exchange capacity (CEC), organic carbon and organic matter (OC, OM), total nitrogen, and the C/N ratio are widely used to describe buffering capacity, nutrient status, and organic matter mineralisation processes (Sharma et al., 2024).These indicators offer a relatively stable representation of soil conditions, although they remain influenced by climate, texture, and fertilization practices.

Biological and biochemical indicators represent the most dynamic component of soil assessment, providing direct information on metabolic status and soil functionality.

Microbial Biomass Carbon (MBC) quantifies the active microbial biomass and is highly sensitive to changes in management, while Dehydrogenase Activity (DHA) reflects the intensity of oxidative–reductive processes associated with microbial metabolism (Chaudhry et al., 2024). Total hydrolytic activity, assessed through Fluorescein Diacetate Hydrolase (FDA), and catalase activity (CAT), associated with hydrogen peroxide decomposition and oxidative stress regulation, are widely used to evaluate functional soil responses to agronomic management practices (Singh et al., 2024). Microbial respiration and the metabolic quotient (qCO_2) provide insight into microbial energy-use efficiency, with elevated values generally reflecting physiological stress, substrate limitation, or reduced metabolic efficiency (Gaudel et al., 2024; Semenov et al., 2025).

Alongside microbial parameters, soil fauna biodiversity provides a key indicator of ecological quality. The QBS-ar index, based on the diversity and adaptation of soil arthropods, is a well-established metric for assessing ecological status and disturbance levels (Parisi et al., 2005). High values indicate stable systems with good ecological quality, whereas low values reflect biologically degraded conditions.

Finally, advanced biochemical indicators, such as the composition of humic fractions, organic matter stability, and the quality of organo-mineral complexes, offer crucial insights into soil capacity for long-term carbon stabilisation and pedological multifunctionality (Lehmann & Kleber, 2015; Sokol et al., 2022).

Integrating physical, chemical, and biological indicators enables the development of multi-indicator assessment frameworks and composite indices to support agroecological planning and regenerative soil management, thereby enhancing agroecosystem resilience and ecosystem service provision, including carbon sequestration (Sharma et al., 2024; Ahmad et al., 2024; Trivedi et al., 2023).

1.3 Sustainable Management and Circular Fertilization Strategies

Sustainable soil management is a central component in ensuring long-term productivity and agroecosystem resilience, particularly in Mediterranean regions characterised by low organic matter content, high summer mineralisation rates, and recurrent water stress. The intensive agriculture that expanded throughout the twentieth century to meet increasing food demand relied heavily on deep tillage, monocropping, and highly soluble chemical inputs, particularly mineral fertilizers such as NPK. Although these inputs initially

increased yields, their prolonged use produced significant collateral effects, including soil acidification, salt accumulation, nutrient imbalances, loss of organic matter, reductions in microbial biomass, and a progressive dependency on synthetic nutrients (Sharma et al., 2024; Muscolo et al., 2022).

Repeated applications of highly soluble NPK fertilizers stimulate rapid mineralisation processes but do not contribute to building stable organic pools, thereby reducing the soil's capacity to retain nutrients and support biological activity over the long term. Moreover, the low nitrogen-use efficiency typical of intensive systems (with leaching and volatilisation losses often exceeding 40–60% of applied N) contributes to water pollution, eutrophication, and emissions of greenhouse gases such as N_2O, a potent climate forcer (Smith et al., 2020). Excess phosphorus from mineral fertilizers saturates adsorption sites, increasing the risk of runoff and contamination of aquatic ecosystems (Withers et al., 2018). These processes compromise essential functions, including hydrological regulation, nutrient cycling, and carbon sequestration, ultimately reducing the soil's capacity to withstand climatic and ecological stress. For these reasons, reducing dependency on NPK and on highly soluble chemical inputs is now a scientific and political priority. Transitioning towards systems based on circular fertilization and renewable organic matrices allows not only the mitigation of the environmental impacts of intensive agriculture but also the strengthening of soil biological processes, improving nutrient-use efficiency and reducing the vulnerability of Mediterranean agroecosystems.

In this context, fertilization with fertilizers produced from wastes emerges as a regenerative paradigm grounded in the valorisation of renewable organic resources and the closure of biogeochemical cycles. Compost, vermicompost, digestates and other processed agro-industrial residues contribute to soil improvement through complementary and interconnected pathways: the stabilisation of organic inputs during biological treatments enhances their suitability as soil amendments, while the persistence of structurally complex organic components supports the gradual incorporation of organic matter into soil matrices, promoting long-term carbon retention and functional soil development (Lehmann & Kleber, 2015; Niedzialkoski et al., 2021). These materials improve porosity, water retention, and soil structure while simultaneously enhancing biodiversity and biological functionality.

Circular fertilization is fully aligned with European ecological-transition strategies,

including the Green Deal, the Farm to Fork Strategy, and the National Recovery and Resilience Plan (NRRP), which promote low-emission production models, resource-use efficiency, and soil regeneration (FAO, 2021; Heyl et al., 2023). The use of organic matrices reduces reliance on synthetic fertilizers, limits the accumulation of reactive nutrients in ecosystems, and contributes to mitigating climate-altering emissions.

The effectiveness of bio-based fertilization is further enhanced when integrated with diversified crop rotations, cover crops, and conservation practices. Cover crops protect soil from erosion, improve infiltration and water availability, and promote soil carbon accumulation, thereby supporting soil biological functioning and crop productivity (Vendig et al., 2023; Blanco-Canqui et al., 2022). Complex and integrated rotations reduce soil fatigue and enhance the complexity and functional diversity of soil microbial networks, strengthening enzymatic processes and biogeochemical stability at the agroecosystem level (Liang et al., 2025; Kong et al., 2025). Meanwhile, reduced tillage and no-tillage practices preserve pore continuity, enhance aggregate stability, and create favourable conditions for soil micro- and mesofauna development, thereby increasing agroecosystem resilience under water-stress conditions (Betancur-Corredor et al., 2022).

The synergistic integration of organic amendments, cover crops, diversified rotations, and conservation tillage promotes agroecologically intensified systems in which soil fertility is increasingly sustained by internal biological processes. Recent evidence shows that vegetation complexity and no-till management enhance soil fauna diversity and biological activity, thereby strengthening the functional resilience of agroecosystems under variable environmental conditions (Mamabolo et al., 2024).

Sustainable soil management and the adoption of circular fertilization strategies are fundamental tools to sustain long-term agricultural productivity, reduce environmental impacts, and promote more resilient and ecologically functional Mediterranean agroecosystems.

1.4 Soil Biodiversity and Ecosystem Functions

Soil biodiversity is a structural and functional component essential for maintaining ecological quality and agroecosystem resilience, playing a particularly critical role in Mediterranean environments characterised by low organic matter content, high hydroclimatic variability, and recurrent water stress. The living component of soil, comprising microorganisms, micro- and mesofauna, roots, and rhizosphere complexes,

sustains pedological functioning through the regulation of biogeochemical processes, stabilisation of soil structure, turnover of organic matter, and modulation of nutrient availability (FAO et al., 2020; Lehmann et al., 2020). This biological complex operates as a self-regulating system capable of integrating environmental changes and anthropogenic pressures.

In Mediterranean systems, pronounced seasonal thermo-hydric oscillations generate marked cyclical patterns in microbial activity: during summer, high temperatures and low soil moisture suppress decomposition and mineralisation, whereas the first autumn rains rapidly reactivate microbial biomass and enzymatic processes. This behaviour is consistent with hierarchical models of pulse dynamics in dryland ecosystems, where episodic wetting events trigger bursts of microbial and biogeochemical activity following drought (Collins et al., 2014). Recent empirical evidence further shows that drying duration and the frequency of rewetting events significantly influence soil respiration, aggregate stability, and nutrient availability through mechanisms linked to microbial reactivation after dry periods (Zhang et al., 2022). The resilience of these cycles is closely associated with the functional diversity, and particularly the network complexity of soil microbial communities, which underpin soil multifunctionality by providing metabolic redundancy and enabling the rapid recovery of key ecosystem processes following physical or climatic disturbances (Chen et al., 2023; Xiao et al., 2025).

Soil fauna contributes complementarily to system functionality. Microarthropods such as mites and springtails, highly sensitive to desiccation and mechanical disturbance, reflect the ecological condition of soils: they are drastically reduced in intensively tilled systems but show higher abundances under conservative orchard and arable management. The QBS-ar index, originally developed to assess soil biological quality through microarthropod communities, effectively captures this sensitivity and has proven to be a robust tool for detecting changes induced by agronomic practices and anthropogenic disturbance across a wide range of agroecosystems (Parisi et al., 2005; Menta et al., 2018; Ari et al., 2023). Likewise, earthworms and other macro-detritivores act as ecosystem engineers, improving porosity, aggregation, and water circulation, critical elements in soils characterised by structural instability.

Microbial and faunal biodiversity also plays a key role in stabilising soil structure. In many Mediterranean soils, characterised by sandy-loam or clay-calcareous textures and limited

organic matter, the microbial production of extracellular polymeric substances (EPS) contributes to the formation of stable aggregates that enhance resistance to intense rainfall and summer drought, within pedological contexts intrinsically prone to structural instability (Six et al., 2004; Zdruli & Miano, 2024). Regular inputs of organic matrices (compost, vermicompost, and agro-industrial residues) further stimulate soil biological processes by promoting decomposer microbial communities involved in organo-mineral complex formation, thereby improving the soil's capacity to retain water and nutrients, an essential aspect for Mediterranean crops exposed to recurrent water deficits (Muscolo et al., 2021; Karaş, 2025).

Soil biodiversity also regulates biotic processes occurring in the rhizosphere, supporting ecological balance between beneficial and potentially harmful organisms. In Mediterranean horticultural systems, exposed to high environmental pressure and frequent moisture fluctuations, well-structured microbial communities enhance soil functional stability and strengthen plant capacity to maintain balanced metabolism. This occurs through a combination of mechanisms, including competition for resources and microhabitats, modification of rhizosphere physico-chemical conditions, production of regulatory metabolites, and stimulation of plant defence responses, that help suppress opportunistic organisms and promote a root environment conducive to plant growth, thereby contributing to soil and ecosystem multifunctionality (Nikitin et al., 2022; Chen, 2024). Conversely, biological simplification driven by monoculture, repeated tillage, and highly soluble mineral inputs reduces the complexity of soil food webs, weakening the soil's self-regulating capacity and increasing crop vulnerability to biotic stress, especially under Mediterranean climates already prone to extreme thermo-hydric fluctuations.

In recent years, the application of omics approaches, including metagenomics, transcriptomics, proteomics, and metabolomics, has significantly improved knowledge of functional biodiversity in Mediterranean soils. Evidence from agricultural systems indicates that microbial resilience to thermal and hydric stress is closely linked to functional traits involved in osmotic regulation, oxidative stress mitigation, and organic matter decomposition, which support the recovery of soil processes after disturbance. In parallel, recent studies integrating biological, chemical, and physical indicators through data-driven approaches demonstrate that soil multifunctionality and resilience under climate change can be effectively predicted by combining multiple functional attributes

(Hu et al., 2024; Gaudel et al., 2024). Integrating omics-derived information with traditional soil indicators therefore opens new perspectives for developing predictive models of soil functionality and management strategies grounded in the principle of biological multifunctionality.

Overall, soil biodiversity in Mediterranean systems is not merely an ecological attribute but a true functional infrastructure that sustains fertility, nutrient-use efficiency, climate resilience, and regenerative capacity. Promoting the conservation and restoration of edaphic biodiversity strengthens soil quality and ensures continuity of essential ecosystem functions, contributing to the transition towards sustainable and climate-resilient agroecological systems.

1.5 Integration of Innovative Assessment Methods

Evaluating soil quality today requires an integrated, process-based approach grounded in an understanding of the physical, chemical, and biological mechanisms that regulate soil system functioning. The inherent complexity of soil, an extremely heterogeneous environment where mineral particles, organic matter, microorganisms, and soil fauna interact, renders diagnostic approaches based solely on static parameters such as pH, texture, or nutrient content insufficient (Lehmann & Kleber, 2015; El Behairy et al., 2024). To capture the true multifunctionality of soil and its resilience, traditional analytical methods must be complemented with biological indicators, enzymatic assays, and rapid monitoring tools.

Conventional chemical analyses remain essential for characterising potential fertility, as they provide information on nutrient availability, cation exchange capacity, organic matter content, and stoichiometric ratios; these indicators are among the most sensitive and widely used parameters for assessing soil quality under varying land uses and management practices (Abshiba, 2025). However, these properties respond slowly to changes in agricultural management and do not describe the soil's ability to support active biogeochemical processes. This limitation underpins the increasing importance of biological and enzymatic indicators, which provide direct measures of microbial activity, organic matter decomposition, and nutrient dynamics. Dehydrogenase activity (DHA) reflects microbial respiratory processes and responds rapidly to changes in organic matter quality or water stress; fluorescein diacetate hydrolase (FDA) measures the soil's overall

degradative potential and is particularly sensitive to organic fertilization; catalase activity indicates the soil system's capacity to detoxify reactive oxygen species and acts as an early signal of functional stress or recovery (Kaur & Kaur, 2021; Sánchez-Monedero et al., 2008; Kunito et al., 2024; Revina et al., 2024). Alongside Microbial Biomass Carbon (MBC) and basal respiration, these indicators directly describe the metabolic status of soil microbial communities and provide robust information on microbial functioning and edaphic vitality, supporting the assessment of soil health under different agricultural practices and environmental stressors, including rapid drying and rewetting cycles typical of Mediterranean systems (Semenov et al., 2025).

Alongside laboratory-based methods, recent years have seen the rapid expansion of rapid techniques and portable diagnostic tools for in-field assessment of soil health. Approaches such as standardized rapid tests and semi-quantitative evaluations enable visual and interpretative assessment of structure, friability, aggregation, macrofauna presence, and root activity, providing immediate feedback on soil physical and biological conditions and allowing early detection of compaction, degradation, or loss of biological vitality (Jordán Vidal, 2023; Reijneveld & Oenema, 2025 At the same time, advances in digital technologies (such as miniaturised Vis–NIR spectroscopy, portable multispectral sensors, and electrochemical probes) have made it possible to estimate key soil parameters, including organic carbon, texture, moisture, and aggregate stability, directly in the field with high spatial resolution. On-the-go sensing platforms mounted on agricultural machinery enable the acquisition of thousands of measurements along continuous transects, enhancing the capacity to monitor soil spatial variability and supporting precision-management models (Kok et al., 2024; Reyes & Ließ, 2023). These techniques are particularly effective in Mediterranean agroecosystems, characterised by strong topographic heterogeneity, high hydroclimatic variability, and structurally fragile soils.

The integration of chemical, biological, and enzymatic indicators with visual observations and rapid diagnostic technologies represents one of the most effective approaches currently available for assessing soil quality in a dynamic and predictive manner. Visual assessments guide immediate agronomic decisions; portable tools enable high-resolution spatial diagnosis; enzymatic assays describe microbial metabolism; and chemical analyses provide the interpretative foundation of the system. This multi-layered approach is strongly promoted by recent European soil-health strategies and is particularly relevant in

Mediterranean environments, where resilience to climate change, regeneration of biological functionality, and efficient nutrient use are essential for agricultural sustainability and the planning of circular and agroecological production systems.

Reference

Abshiba, K., Sharma, V. K., Kumar, P., Syam, S., Barnwal, P. P., & Kumar, S. (2025). A comprehensive review of soil quality indicators under diverse land uses in rainfed areas. Discover Soil, 2(1), 91. https://doi.org/10.1007/s44378-025-00109-5

Ahmad, R., Hassan, A., Sheikh, T., Shakil, M., Nisa, R.-U., Alkilayh, O. A., Hamid, B., Baba, Z., & Sayyed, R. Z. (2024). Assessment of the sustainability of land use systems through sensitive indicators of soil quality and biophysical properties in temperate ecosystem. Egyptian Journal of Soil Science, 64(4), 1389–1406. https://doi.org/10.21608/ejss.2024.294037.1780

Ari, P. M. S., Rahadian, R., & Hadi, M. (2023). Comparison of soil fertility levels of coffee-based agroforestry land cultivation types based on the QBS-ar soil microarthropod index. DIVERSITAS HAYATI, 1(2), 1–6. https://doi.org/10.30631/12.1-6

Betancur-Corredor, B., Lang, B., & Russell, D. J. (2022). Reducing tillage intensity benefits the soil micro- and mesofauna in a global meta-analysis. European Journal of Soil Science, 73(6), e13321. https://doi.org/10.1111/ejss.13321

Bhaduri, D., Sihi, D., Bhowmik, A., Verma, B. C., Munda, S., & Dari, B. (2022). A review on effective soil health bio-indicators for ecosystem restoration and sustainability. Frontiers in Microbiology, 13. https://doi.org/10.3389/fmicb.2022.938481

Bhattacharyya, T. (2024). Soil Carbon Footprints and Climate-smart Soils. Current Science, 126(5), 548. https://doi.org/10.18520/cs/v126/i5/548-554

Blanco-Canqui, H., Ruis, S. J., Holman, J. D., Creech, C. F., & Obour, A. K. (2022). Can cover crops improve soil ecosystem services in water-limited environments? A review. Soil Science Society of America Journal, 86(1), 1–18. https://doi.org/10.1002/saj2.20335

Carswell, A. M., Willcock, S., Blackwell, M. S. A., Upadhayay, H. R., Harris, P., McAuliffe, G., Neal, A. L., Rivero, M. J., Cardenas, L. M., Haefele, S. M., Whitmore, A. P., Dearing, J. A., Zhang, F., Farrell, M., Bauters, M., Boeckx, P., Silva, Y. J. A. B. da, Frimpong, K. A., & Collins, A. L. (2025). Agricultural practices can threaten soil resilience through changing feedback loops. Npj Sustainable Agriculture, 3(1), 56.

https://doi.org/10.1038/s44264-025-00098-6

Chaudhry, H., Vasava, H. B., Chen, S., Saurette, D., Beri, A., Gillespie, A., Biswas, A., Chaudhry, H., Vasava, H. B., Chen, S., Saurette, D., Beri, A., Gillespie, A., & Biswas, A. (2024). Evaluating the Soil Quality Index Using Three Methods to Assess Soil Fertility. Sensors, 24(3). https://doi.org/10.3390/s24030864

Chen, Q., Song, Y., An, Y., Lu, Y., Zhong, G., Chen, Q., Song, Y., An, Y., Lu, Y., & Zhong, G. (2024). Soil Microorganisms: Their Role in Enhancing Crop Nutrition and Health. Diversity, 16(12). https://doi.org/10.3390/d16120734

Chen, Y., Chi, J., Lu, X., Cai, Y., Jiang, H., Zhang, Q., & Zhang, K. (2023). Fungal-bacterial composition and network complexity determine soil multifunctionality during ecological restoration. CATENA, 230, 107251. https://doi.org/10.1016/j.catena.2023.107251

Collins, S. L., Belnap, J., Grimm, N. B., Rudgers, J. A., Dahm, C. N., D'Odorico, P., Litvak, M., Natvig, D. O., Peters, D. C., Pockman, W. T., Sinsabaugh, R. L., & Wolf, B. O. (2014). A Multiscale, Hierarchical Model of Pulse Dynamics in Arid-Land Ecosystems. Annual Review of Ecology, Evolution, and Systematics, 45(Volume 45, 2014), 397–419. https://doi.org/10.1146/annurev-ecolsys-120213-091650

Denning, G. (2025). Sustainable intensification of agriculture: The foundation for universal food security. Npj Sustainable Agriculture, 3(1), 7. https://doi.org/10.1038/s44264-025-00047-3

El Behairy, R. A., El Arwash, H. M., El Baroudy, A. A., Ibrahim, M. M., Mohamed, E. S., Kucher, D. E., Shokr, M. S., Behairy, R. A. E., Arwash, H. M. E., Baroudy, A. A. E., Ibrahim, M. M., Mohamed, E. S., Kucher, D. E., & Shokr, M. S. (2024). How Can Soil Quality Be Accurately and Quickly Studied? A Review. Agronomy, 14(8). https://doi.org/10.3390/agronomy14081682

FAO. (2021). Global assessment of soil pollution. https://doi.org/10.4060/cb4894en

FAO. (2023). FAOSTAT Statistical Database. Food and Agriculture Organization of the United Nations. https://www.fao.org/faostat/en/#home

FAO, ITPS, GSBI, SCBD, & EC. (2020). State of knowledge of soil biodiversity—Status, challenges and potentialities. Report 2020 (1st edn). FAO. https://doi.org/10.4060/cb1928en

Gaudel, G., Xing, L., Raseduzzaman, M., Poudel, M., Dong, W., & Hu, C. (2024). Soil

microbes, carbon, nitrogen, and the carbon to nitrogen ratio indicate priming effects across terrestrial ecosystems. Journal of Soils and Sediments, 24(1), 307–322. https://doi.org/10.1007/s11368-023-03609-5

Heyl, K., Ekardt, F., Roos, P., & Garske, B. (2023). Achieving the nutrient reduction objective of the Farm to Fork Strategy. An assessment of CAP subsidies for precision fertilization and sustainable agricultural practices in Germany. Frontiers in Sustainable Food Systems, 7. https://doi.org/10.3389/fsufs.2023.1088640

Hu, X., Xie, Y., Zhou, Q., & Mu, L. (2024). Machine learning approach for Studying the multifunctionality of soil against global climate changes. Ecological Indicators, 169, 112772. https://doi.org/10.1016/j.ecolind.2024.112772

Intergovernmental Panel On Climate Change (IPCC). (2022). Climate Change 2022: Impacts, Adaptation and Vulnerability: Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change (1st edn). Cambridge University Press. https://doi.org/10.1017/9781009325844

IPBES. (2019). Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. Zenodo. https://doi.org/10.5281/zenodo.6417333

Janzen, H. H., Janzen, D. W., & Gregorich, E. G. (2021). The 'soil health' metaphor: Illuminating or illusory? Soil Biology and Biochemistry, 159, 108167. https://doi.org/10.1016/j.soilbio.2021.108167

Jordán Vidal, M. M. (2023). Criteria for Assessing the Environmental Quality of Soils in a Mediterranean Region for Different Land Use. Soil Systems, 7(3). https://doi.org/10.3390/soilsystems7030075

Karaş, E. (2025). Sustainable Soil and Water Management in Arid Climates in the Mediterranean Climate Zone. In Beyond Rain—The Future of Irrigation in a Changing Climate. IntechOpen. https://doi.org/10.5772/intechopen.1009836

Kaur, J., & Kaur, G. (2021). Dehydrogenase Activity as a Biological Indicator of Soil Health. Chemical Science Review and Letters, 10(39), 326–329.

Kok, M., Sarjant, S., Verweij, S., Vaessen, S. F. C., & Ros, G. H. (2024). On-site soil analysis: A novel approach combining NIR spectroscopy, remote sensing and deep learning. Geoderma, 446, 116903. https://doi.org/10.1016/j.geoderma.2024.116903

Kong, W., Yao, Y., Qiu, L., Shao, M., & Wei, X. (2025). Crop rotation enhances soil microbial

network complexity and functionality: A meta-analysis. Applied Soil Ecology, 216, 106511. https://doi.org/10.1016/j.apsoil.2025.106511

Kunito, T., Moro, H., Mise, K., Sawada, K., Otsuka, S., Nagaoka, K., & Fujita, K. (2024). Ecoenzymatic stoichiometry as a temporally integrated indicator of nutrient availability in soils. Soil Science and Plant Nutrition, 70(4), 246–269. https://doi.org/10.1080/00380768.2024.2341669

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. Nature Reviews Earth & Environment, 1(10), 544–553. https://doi.org/10.1038/s43017-020-0080-8

Liang, X., Yu, S., Ju, Y., Wang, Y., Yin, D., Liang, X., Yu, S., Ju, Y., Wang, Y., & Yin, D. (2025). Integrated Management Practices Foster Soil Health, Productivity, and Agroecosystem Resilience. Agronomy, 15(8). https://doi.org/10.3390/agronomy15081816

Mamabolo, E., Pryke, J. S., & Gaigher, R. (2024). Soil fauna diversity is enhanced by vegetation complexity and no-till planting in regenerative agroecosystems. Agriculture, Ecosystems & Environment, 367, 108973. https://doi.org/10.1016/j.agee.2024.108973

Menta, C., Conti, F. D., Pinto, S., & Bodini, A. (2018). Soil Biological Quality index (QBS-ar): 15 years of application at global scale. Ecological Indicators, 85, 773–780. https://doi.org/10.1016/j.ecolind.2017.11.030

Montanarella, L., & Panagos, P. (2021). Soil Security for the European Union. Soil Security, 4, 100009. https://doi.org/10.1016/j.soisec.2021.100009

Muscolo, A., Marra, F., Canino, F., Maffia, A., Mallamaci, C., & Russo, Mt. (2022). Growth, nutritional quality and antioxidant capacity of lettuce grown on two different soils with sulphur-based fertilizer, organic and chemical fertilizers. Scientia Horticulturae, 305, 111421. https://doi.org/10.1016/j.scienta.2022.111421

Muscolo, A., Romeo, F., Marra, F., & Mallamaci, C. (2021). Recycling agricultural, municipal and industrial pollutant wastes into fertilizers for a sustainable healthy food production. Journal of Environmental Management, 300, 113771. https://doi.org/10.1016/j.jenvman.2021.113771

Niedzialkoski, R. K., Marostica, R., Damaceno, F. M., Costa, L. A. de M., & Costa, M. S. S. de M. (2021). Combination of biological processes for agro-industrial poultry waste

management: Effects on vermicomposting and anaerobic digestion. Journal of Environmental Management, 297, 113127. https://doi.org/10.1016/j.jenvman.2021.113127

Nikitin, D. A., Semenov, M. V., Chernov, T. I., Ksenofontova, N. A., Zhelezova, A. D., Ivanova, E. A., Khitrov, N. B., & Stepanov, A. L. (2022). Microbiological Indicators of Soil Ecological Functions: A Review. Eurasian Soil Science, 55(2), 221–234. https://doi.org/10.1134/S1064229322020090

Panagos, P., Broothaerts, N., Ballabio, C., Orgiazzi, A., De Rosa, D., Borrelli, P., Liakos, L., Vieira, D., Van Eynde, E., Arias Navarro, C., Breure, T., Fendrich, A., Köninger, J., Labouyrie, M., Matthews, F., Muntwyler, A., Jimenez, J. M., Wojda, P., Yunta, F., … Jones, A. (2024). How the EU Soil Observatory is providing solid science for healthy soils. European Journal of Soil Science, 75(3), e13507. https://doi.org/10.1111/ejss.13507

Parisi, V., Menta, C., Gardi, C., Jacomini, C., & Mozzanica, E. (2005). Microarthropod communities as a tool to assess soil quality and biodiversity: A new approach in Italy. Agriculture, Ecosystems & Environment, 105(1), 323–333. https://doi.org/10.1016/j.agee.2004.02.002

Reijneveld, J. A., & Oenema, O. (2025). Rapid Soil Tests for Assessing Soil Health. Applied Sciences, 15(15). https://doi.org/10.3390/app15158669

Revina, S., Minnikova, T., Ruseva, A., Kolesnikov, S., & Kutasova, A. (2024). Catalase activity as a diagnostic indicator of the health of oil-contaminated soils after remediation. Environmental Monitoring and Assessment, 196(5), 449. https://doi.org/10.1007/s10661-024-12604-3

Reyes, J., Ließ, M., Reyes, J., & Ließ, M. (2023). On-the-Go Vis-NIR Spectroscopy for Field-Scale Spatial-Temporal Monitoring of Soil Organic Carbon. Agriculture, 13(8). https://doi.org/10.3390/agriculture13081611

Sánchez-Monedero, M. A., Mondini, C., Cayuela, M. L., Roig, A., Contin, M., & De Nobili, M. (2008). Fluorescein diacetate hydrolysis, respiration and microbial biomass in freshly amended soils. Biology and Fertility of Soils, 44(6), 885–890. https://doi.org/10.1007/s00374-007-0263-1

Semenov, M. V., Zhelezova, A. D., Ksenofontova, N. A., Ivanova, E. A., Nikitin, D. A., Semenov, V. M., Semenov, M. V., Zhelezova, A. D., Ksenofontova, N. A., Ivanova, E. A., Nikitin, D. A., & Semenov, V. M. (2025). Microbiological Indicators for Assessing the

Effects of Agricultural Practices on Soil Health: A Review. Agronomy, 15(2). https://doi.org/10.3390/agronomy15020335

Sharma, S., Kaur, G., Singh, P., Ghuman, R. S., Singh, P., & Vyas, P. (2024). Distinct changes in soil organic matter quality, quantity and biochemical composition in response to land-use change to diverse cropping systems and agroforestry in north-western India. Agroforestry Systems, 98(4), 1049–1073. https://doi.org/10.1007/s10457-024-00976-x

Shepherd, G. (2000). Visual soil assessment: Field guide for cropping [Practice tool]. Landcare Research. https://orgprints.org/id/eprint/30582/

Singh, N., . A., Maurya, V. K., & Singh, D. (2024). Soil Enzyme Activities as Bioindicators of Soil Health: Implications for Sustainable Agriculture. Journal of Natural Resource Conservation and Management, 5(2), 124–136. https://doi.org/10.51396/ANRCM.5.2.2024.124-136

Six, J., Bossuyt, H., Degryze, S., & Denef, K. (2004). A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research, 79(1), 7–31. https://doi.org/10.1016/j.still.2004.03.008

Smith, P., Soussana, J.-F., Angers, D., Schipper, L., Chenu, C., Rasse, D. P., Batjes, N. H., van Egmond, F., McNeill, S., Kuhnert, M., Arias-Navarro, C., Olesen, J. E., Chirinda, N., Fornara, D., Wollenberg, E., Álvaro-Fuentes, J., Sanz-Cobena, A., & Klumpp, K. (2020). How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. Global Change Biology, 26(1), 219–241. https://doi.org/10.1111/gcb.14815

Sokol, N. W., Whalen, E. D., Jilling, A., Kallenbach, C., Pett-Ridge, J., & Georgiou, K. (2022). Global distribution, formation and fate of mineral-associated soil organic matter under a changing climate: A trait-based perspective. Functional Ecology, 36(6), 1411–1429. https://doi.org/10.1111/1365-2435.14040

Trivedi, P., Leach, J. E., Tringe, S. G., Sa, T., & Singh, B. K. (2020). Plant–microbiome interactions: From community assembly to plant health. Nature Reviews Microbiology, 18(11), 607–621. https://doi.org/10.1038/s41579-020-0412-1

Vendig, I., Guzman, A., De La Cerda, G., Esquivel, K., Mayer, A. C., Ponisio, L., & Bowles, T. M. (2023). Quantifying direct yield benefits of soil carbon increases from cover cropping. Nature Sustainability, 6(9), 1125–1134. https://doi.org/10.1038/s41893-023-

01131-7

Wang, N., & Zhang, T. (2024). Soil pore structure and its research methods: A review. Soil and Water Research, 19(1), 1–24. https://doi.org/10.17221/64/2023-SWR

Withers, P. J. A., Rodrigues, M., Soltangheisi, A., de Carvalho, T. S., Guilherme, L. R. G., Benites, V. de M., Gatiboni, L. C., de Sousa, D. M. G., Nunes, R. de S., Rosolem, C. A., Andreote, F. D., Oliveira, A. de, Coutinho, E. L. M., & Pavinato, P. S. (2018). Transitions to sustainable management of phosphorus in Brazilian agriculture. Scientific Reports, 8(1), 2537. https://doi.org/10.1038/s41598-018-20887-z

Xiao, Y., Wang, J., Wang, B., Fan, B., & Zhou, G. (2025). Soil microbial network complexity predicts soil multifunctionality better than soil microbial diversity during grassland-farmland-shrubland conversion on the Qinghai-Tibetan Plateau. Agriculture, Ecosystems & Environment, 379, 109356. https://doi.org/10.1016/j.agee.2024.109356

Zhang, Z., Wang, D., & Li, M. (2022). Soil respiration, aggregate stability and nutrient availability affected by drying duration and drying-rewetting frequency. Geoderma, 413, 115743. https://doi.org/10.1016/j.geoderma.2022.115743

PhD Thesis Objectives

The rising global demand for food, feed, energy, and biomass is intensifying pressure on agricultural soils and accelerating degradation processes that threaten productivity, ecological stability, and human well-being. In Mediterranean systems, these phenomena are amplified by low organic matter content, high summer mineralisation rates, increasing aridity, and strong climatic variability. Soil quality degradation, including organic matter decline, simplification of biotic communities, and loss of filtering and regulatory functions, compromises food security, increases the risk of water contamination, and weakens the ability of agroecosystems to sustain ecological resilience (FAO, 2021; Ferreira et al., 2024).

The overall objective of this PhD research project is to evaluate, through an integrated approach, the effects of sustainable fertilizers derived from agro-industrial residues on soil quality, soil biodiversity, and ecosystem functionality in Mediterranean horticultural systems. The adopted framework combines chemical, physical, and biological indicators to interpret soil responses to different fertilization regimes, privileging a process-based and dynamic reading of biogeochemical cycles over assessments grounded solely in static properties (Lehmann et al., 2020; Fan, 2025). This integration enables the analysis of how management practices influence not only immediate fertility but also the long-term resilience of the soil system.

Attention is dedicated to the QBS-ar index, an established indicator of soil biological quality based on the functional structure of soil macrofauna. Owing to its sensitivity to anthropogenic disturbance and management changes, QBS-ar is an effective tool for detecting early variations in soil naturalness and ecological network complexity (Gallese et al., 2025). Its integration with chemical (pH, CEC, organic carbon), biological (microbial biomass, enzymatic activity), and physical (water-holding capacity, structure) parameters enables a multilayered assessment of soil health.

The comparison among matrices of different origin, composition, and stability, such as compost and vermicompost derived from organic and lignocellulosic residues, RecOrgFert PLUS an innovative fertilizer formulated from citrus-processing residues and elemental sulphur, farmyard manure, and NPK a mineral fertilizer, allow understand on a comparative basic how each input modulates soil biological processes, organic matter

dynamics, and the capacity of the soil system to support key ecosystem functions. More stabilised organic matrices are expected to promote carbon accumulation, microbial activity, and functional complexity of soil biota, whereas more soluble inputs tend to affect biogeochemical cycles more rapidly but less persistently, potentially compromising long-term soil functionality, particularly where sulphur dynamics and organic matter interactions play a key role (Muscolo et al., 2022; Zhou et al., 2024). This comparison provides insights into the mechanisms through which different fertilizers contribute to, or potentially impair, the processes that regulate soil quality and soil health in Mediterranean horticultural systems.

The specific objectives of the research were to:

- Evaluate the effects of sustainable and conventional fertilizers on soil chemical fertility, with reference to pH, CEC, organic carbon, organic matter, and macro- and micronutrients.
- Analyse soil biological functionality through enzymatic assays (dehydrogenase, fluorescein diacetate hydrolase, catalase) to characterise microbial vitality and system responses to external inputs.
- Assess the biodiversity of soil macrofauna through the QBS-ar index, integrating these data with chemical and biochemical indicators to describe system resilience and naturalness.
- Compare soil responses to sustainable and conventional fertilizers, identifying positive effects, potential drawbacks, and mechanisms through which ecosystem functionality may be enhanced.
- Integrate chemical, biological, and physical indicators into a unified assessment framework to develop a comprehensive diagnosis of soil health, supporting the design of sustainable management strategies aligned with the principles of agroecology and circular fertilization (Wezel et al., 2025).

Reference

Fan, Y., Zhang, C., Hu, W., Khan, K. S., Zhao, Y., & Huang, B. (2025). Development of soil quality assessment framework: A comprehensive review of indicators, functions, and approaches. Ecological Indicators, 172, 113272.

https://doi.org/10.1016/j.ecolind.2025.113272

FAO. (2021). Global assessment of soil pollution. https://doi.org/10.4060/cb4894en

Ferreira, C. S. S., Keesstra, S., Destouni, G., Solomun, M. K., & Kalantari, Z. (2024). Soil Degradation in the Mediterranean Region: Drivers and Future Trends. In C. S. S. Ferreira, G. Destouni, & Z. Kalantari (Eds), Environmental Sustainability in the Mediterranean Region: Challenges and Solutions (pp. 81–112). Springer International Publishing. https://doi.org/10.1007/978-3-031-64503-7_5

Gallese, F., Gismero-Rodriguez, L., Govednik, A., Giagnoni, L., Lumini, E., Suhadolc, M., Vaccari, F. P., Maienza, A., Gallese, F., Gismero-Rodriguez, L., Govednik, A., Giagnoni, L., Lumini, E., Suhadolc, M., Vaccari, F. P., & Maienza, A. (2025). Soil Microarthropods as Tools for Monitoring Soil Quality: The QBS-ar Index in Three European Agroecosystems. Agriculture, 15(1). https://doi.org/10.3390/agriculture15010089

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., & Rillig, M. C. (2020). The concept and future prospects of soil health. Nature Reviews Earth & Environment, 1(10), 544–553. https://doi.org/10.1038/s43017-020-0080-8

Muscolo, A., Marra, F., Canino, F., Maffia, A., Mallamaci, C., & Russo, Mt. (2022). Growth, nutritional quality and antioxidant capacity of lettuce grown on two different soils with sulphur-based fertilizer, organic and chemical fertilizers. Scientia Horticulturae, 305, 111421. https://doi.org/10.1016/j.scienta.2022.111421

Wezel, A., Marchetti, A., Nichenametla, C. K., Boughamoura, O., Kamilia, K., & Bàrberi, P. (2025). Multiple agroecological practices use and climate change mitigation. A review. Agronomy for Sustainable Development, 45(5), 58. https://doi.org/10.1007/s13593-025-01048-9

Zhou, T., Wang, Z., Lv, Q., Zhang, Y., Tao, S., Ren, X., Gao, H., Gao, Z., & Hu, S. (2024). Sulfur dynamics in saline sodic soils: The role of paddy cultivation and organic amendments. Ecological Indicators, 162, 112014. https://doi.org/10.1016/j.ecolind.2024.112014

Results Organization

The results of this thesis are organised according to a thematic and progressive structure, developed around a series of experimental studies and scientific publications that guide the reader from the general theoretical framework to the experimental evidence concerning the effects of sustainable fertilizers on soil quality. This structure enables coherent integration of chemical, biological, biochemical, and faunal data, providing a multilayered interpretation of soil responses to different fertilization regimes and allowing sensitive detection of early signals of improvement or degradation in Mediterranean agricultural systems.

The diversity of the fertilizing matrices considered, composts and vermicomposts derived from organic and lignocellulosic residues, RecOrgFert PLUS produced from agro-industrial orange waste combined with elemental sulphur, farmyard manure, and mineral NPK, offers the opportunity to systematically compare circular-management strategies with conventional practices. The integration of enzymatic assays, chemical analyses, and the QBS-ar index makes it possible to characterise simultaneously the structural component of the soil, its microbial vitality, and faunal complexity, thereby contributing to an early functional diagnosis of underlying processes.

Papers details

The thesis is organised into four main chapters, each based on an experimental study that has been published or is currently under review:

- **Chapter 2** — Maffia Angela, Marra Federica, Canino Francesco, Battaglia Santo, Mallamaci Carmelo, **Oliva Mariateresa** and Muscolo Adele, (2024).

Humic Substances: Bridging Ecology and Agriculture for a Greener Future. Agronomy https://doi.org/10.3390/agronomy14112657

Journal bibliometric classification of Agronomy: IF 3.3, category agronomy and crop science quartile Q1

Mariateresa Oliva role and contribution in this paper: software.

- **Chapter 3 —** Maffia Angela, Marra Federica, Battaglia Santo, **Oliva Mariateresa**, Mallamaci Carmelo, Muscolo Adele (2024).

Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and

Yield of Red Cabbage and Broccoli. Soil Systems.

https://doi.org/10.3390/soilsystems8020053

Journal bibliometric classification of Soil Systems IF 2.9, category Soil Science quartile Q1.

Mariateresa Oliva role and contribution of this paper: methodology, investigation.

• **Chapter 4 — Oliva Mariateresa,** Marra Federica, Santoro Ludovica, Battaglia Santo, Mallamaci Carmelo, Adele Muscolo (2025)

Circular Fertilization Strategy Using Sulphur with Orange Waste Enhances Soil Health and Broccoli Nutritional and Nutraceutical Quality in Mediterranean Systems. Applied Sciences.

https://doi.org/10.3390/app15169010

Journal bibliometric classification of Applied Sciences: IF 2.7, category Agronomy quartile Q2.

Mariateresa Oliva role and contribution of this paper: validation, investigation, data curation, writing—review and editing, supervision.

• **Chapter 5 — Oliva Mariateresa,** Marra Federica, Santoro Ludovica, Battaglia Santo, Mallamaci Carmelo, Adele Muscolo.

A Multi-Indicator Approach to Soil Quality in Agroecological Systems Based on the Use of Organic Waste within a Circular Economy Model

Mariateresa Oliva role and contribution of this paper: validation, investigation, data curation, writing—review and editing, supervision.

Additional papers

- Maffia Angela, Marra Federica, **Oliva Mariateresa**, Battaglia Santo, Mallamaci Carmelo and Muscolo Adele, (2025).

  Sustainable Soil Management: The Dynamic Impact of Combined Use of Crop Rotation and Fertilizers from Agri-Food and Sulfur Hydrocarbon Refining Processes Waste, Land.

  https://doi.org/10.3390/land14061171

  Journal bibliometric classification of *Land*: IF 3.2, category *Environmental Studies (SSCI)*, quartile Q2.

  Mariateresa Oliva role and contribution of this paper: validation.

- Adele Muscolo, Konstantinos Zoukidis, Evangelos Vergos, Federica A. Marra, Ludovica Santoro, **Mariateresa Oliva**, Carmelo Mallamaci, (2025).

Impacts of Conventional and Agri-Food Waste-Derived Fertilizers on Durum Wheat Yield, Grain Quality, and Soil Health: A Two-Year Field Study in Greece and Southern Italy, Applied Sciences.

https://doi.org/10.3390/app151810292

Journal bibliometric classification of Applied Sciences: IF 2.7, category Agronomy, quartile Q2.

Mariateresa Oliva role and contribution of this paper: software, data curation, writing—original draft preparation, visualization.

- Maffia Angela, Marra Federica, Battaglia Santo, **Oliva Mariateresa**, Mallamaci Carmelo, Muscolo Adele (2024).

  Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli. Soil Systems.

  https://doi.org/10.3390/soilsystems8020053

  Journal bibliometric classification of Soil Systems IF 2.9, category Soil Science quartile Q1.

  Mariateresa Oliva role and contribution of this paper: methodology and investigation.

- Maffia Angela, Marra Federica, Canino Francesco, Battaglia Santo, Mallamaci Carmelo, **Oliva Mariateresa** and Muscolo Adele, (2024).

  Humic Substances from Waste-Based Fertilizers for improved soil fertility. Agronomy

  https://doi.org/10.3390/agronomy14112657

  Journal bibliometric classification of Agronomy: IF 3.3, category agronomy and crop science quartile Q1

  Mariateresa Oliva role and contribution of this paper: formal analysis.

- Maffia Angela, Marra Federica, Celano Giuseppe, **Oliva Mariateresa**, Mallamaci Carmelo, Hussain MI and Muscolo Adele, (2024).

  Exploring the Potential and Obstacles of Agro-industrial Waste-Based Fertilizers, Land.

  https://doi.org/10.3390/land13081166

  Journal bibliometric classification of Land IF 3.2, category Nature and Landscape conservation quartile Q1.

  Mariateresa Oliva role and contribution of this paper: formal analysis.

Chapter 2 - Humic Substances: Bridging Ecology and Agriculture for a Greener Future

Angela Maffia [1], **Mariateresa Oliva** [1], Federica Marra [1], Carmelo Mallamaci [1], Serenella Nardi [2] and Adele Muscolo [1*]

[1]Department of AGRARIA, 'Mediterranea' University of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); mariateresa.oliva@unirc.it (M.O.); federica.marra@unirc.it (F.M.); carmelo.mallamaci@unirc.it (C.M.)

[2] Department di Agronomia, Animali, Alimenti, Risorse Naturali e Ambiente (DAFNAE), Università degli Studi di Padova, 35020 Legnaro, Padova, Italy; serenella.nardi@unipd.it

*Correspondence: amuscolo@unirc.it

Agronomy

https://doi.org/10.3390/agronomy15020410

Received: 30 December 2024

Revised: 23 January 2025

Accepted: 29 January 2025

Published: 6 February 2025

agronomy

MDPI

Review

Humic Substances: Bridging Ecology and Agriculture for a Greener Future

Angela Maffia [1], Mariateresa Oliva [1], Federica Marra [1], Carmelo Mallamaci [1], Serenella Nardi [2] and Adele Muscolo [1,*]

[1] Department of AGRARIA, 'Mediterranea' University of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); mariateresa.oliva@unirc.it (M.O.); federica.marra@unirc.it (F.M.); carmelo.mallamaci@unirc.it (C.M.)

[2] Department di Agronomia, Animali, Alimenti, Risorse Naturali e Ambiente (DAFNAE), Università degli Studi di Padova, 35020 Legnaro, Padova, Italy; serenella.nardi@unipd.it

* Correspondence: amuscolo@unirc.it

Abstract: Humic substances (HSs) are emerging as multifunctional natural catalysts in sustainable agriculture, offering novel opportunities to enhance soil health, plant productivity, and environmental resilience. This review synthesizes recent insights into the chemical diversity, biological mechanisms, and ecological impacts of HSs, presenting a new perspective on their role as dynamic agents in agroecosystems. Derived from decomposed organic matter, HSs regulate critical processes such as nutrient cycling, carbon sequestration, and pollutant detoxification. Unlike plant and microbial biomass, which undergo rapid mineralization due to their active dynamism, HSs exhibit significant resistance to biodegradation, leading to a prolonged residence time in soil that spans years or even centuries. This stability allows HSs to maintain their functional roles over extended periods, contributing to long-term soil health and ecosystem sustainability. Their integration into agricultural systems has demonstrated profound effects, including improved soil structure, increased water retention, and the stimulation of microbial activity, which collectively bolster plant stress tolerance and yield. Notably, it has been proposed that HSs exhibit hormone-like properties, influencing plant signaling pathways to enhance root architecture and nutrient acquisition. Moreover, HSs contribute to environmental remediation by regulating the leaching of heavy metals, mitigating nutrient runoff, and fostering climate resilience. This review highlights the synergistic potential of combining HSs with organic amendments like compost and biochar, positioning HSs as a cornerstone of regenerative farming practices. Addressing challenges such as variability in composition and application methods, the discussion underscores the urgency of developing standardized approaches to harness their full potential. By framing HSs as versatile and adaptive tools, this review paves the way for advancing sustainable agricultural systems while addressing global challenges like food security and climate change.

Keywords: humic substances; sustainable agriculture; soil health; plant biostimulants; environmental resilience

check for updates

Academic Editor: Jiafa Luo

Received: 30 December 2024
Revised: 23 January 2025
Accepted: 29 January 2025
Published: 6 February 2025

Citation: Maffia, A.; Oliva, M.; Marra, F.; Mallamaci, C.; Nardi, S.; Muscolo, A. Humic Substances: Bridging Ecology and Agriculture for a Greener Future. *Agronomy* **2025**, *15*, 410. https://doi.org/10.3390/agronomy15020410

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

1.1. Humic Substances Overview

This review synthesizes data from a broad range of peer-reviewed studies, meta-analyses, and recent advancements in soil chemistry and agricultural sciences, focusing on the role of humic substances (HSs) in soil ecosystems. The selected literature encompasses

seminal works that laid the foundation for understanding the chemical and biological properties of HSs, as well as more recent studies exploring their applications in enhancing soil health and plant productivity. Comprehensive databases, including Web of Science, Scopus, and Google Scholar, were utilized to gather relevant and methodologically rigorous studies.

The body of literature spans several decades, from the foundational research of the late 20th century to contemporary contributions that address the multifaceted roles of HSs in agricultural sustainability. In total, 136 articles were analyzed, with the selection process guided by keywords such as "Humic Substances", "Humic Acids" (HAs), "Fulvic Acids" (FAs), "Humin (HU), and "Organic fertilizers from HS". This review thus offers a holistic perspective, combining historical insights with the latest developments in the field. Emphasis is placed on the influence of HSs on soil health, including their effects on soil structure, water retention, nutrient cycling, and microbial activity. Additionally, the review examines their impact on plant growth, stress resilience, and productivity (Figure 1). The potential applications of HSs in organic farming and their broader ecological implications, including carbon sequestration and biodiversity enhancement, are also discussed.

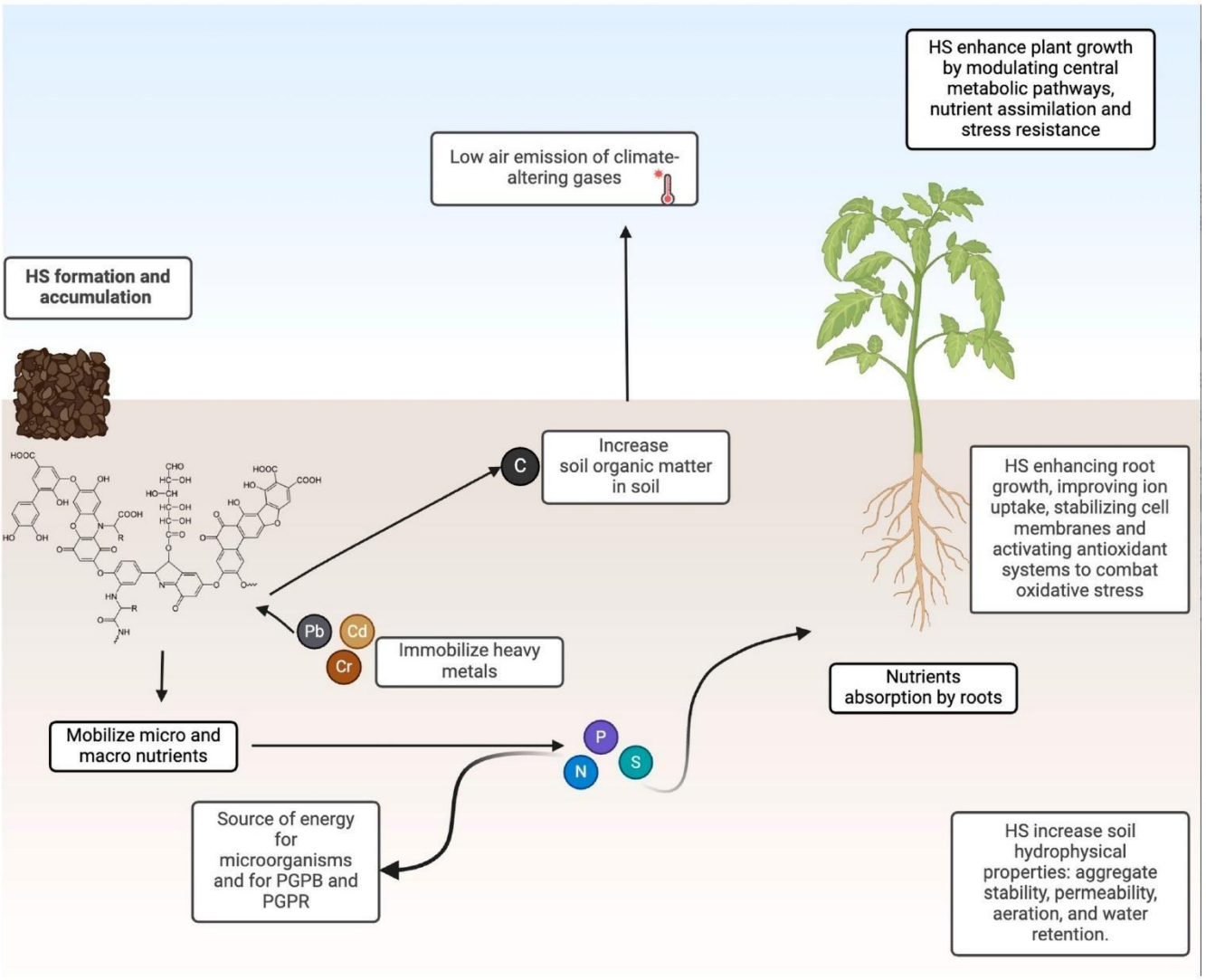


Figure 1. An overview of the role of HSs in soil, both for plants and the environment, in the context of sustainable agriculture.

Finally, the review highlights the challenges and future directions for the utilization of HSs in agricultural practices, particularly in the context of global environmental changes and the transition toward more sustainable agricultural systems.

1.2. The Role of HSs in Sustainable Agriculture

Sustainable agriculture is fundamental to addressing the growing challenges of global food security, soil degradation, and environmental sustainability in the face of climate change and population growth. By 2050, the global population is expected to reach 9.7 billion, necessitating a 70% increase in food production [1]. Unlike conventional farming, which often leads to soil erosion, water pollution, and biodiversity loss, sustainable agriculture integrates ecological principles to maintain productivity while safeguarding natural resources [2]. Core objectives include enhancing soil fertility, minimizing pollution, promoting biodiversity, and ensuring long-term economic viability for farmers [3,4]. Sustainable practices such as crop rotation, conservation tillage, organic farming, and agroforestry improve soil health and resilience [5]. Precision agriculture, which leverages digital technologies and data analytics, also supports sustainable goals by optimizing resource use and reducing environmental impact [6]. In addition, integrated nutrient management strategies, including the use of organic amendments like HSs, play a critical role in enhancing soil structure and nutrient availability [7–9]. HSs, derived from decomposed organic matter, have been identified as key contributors to sustainable soil management. They improve cation exchange capacity (CEC), water retention, and microbial diversity, which are essential for maintaining soil productivity under sustainable agricultural systems (Figure 1) [10]. Recent studies highlighted that HSs can also enhance plant resilience to drought and nutrient stress, making them particularly valuable in the context of climate change [11]. The action of HSs on plants occurs in two ways—indirectly, as a modification of soil properties, and as a direct impact on physical and metabolic plant processes. Furthermore, Nardi et al. [12] demonstrated how the HSs and their fractions positively affect primary and secondary metabolic pathways of plants both in laboratory and field studies. The considerable effect of HSs on lateral root induction and root hair growth might be caused by changes in energetic and nitrogen pathways and transport across membranes. HSs influence plant nutrition and development through closely related mechanisms that are hormone-dependent.

Adopting sustainable agricultural practices not only secures food production but also supports ecosystem services, such as pollination, pest regulation, and nutrient cycling, which are fundamental for long-term productivity [13,14]. Therefore, integrating organic matter and HSs into farming systems represents a practical strategy to enhance both soil health and agricultural sustainability [15]. Policymakers, researchers, and farmers must collaborate to implement these practices on a global scale to ensure a resilient and sustainable food system for future generations [16]. The application of HSs in agriculture has been shown to enhance plant growth, root development, and resistance to abiotic stress, making them essential for sustainable practices [17]. As a pivotal ecological force, HSs improve soil health, which is fundamental for maintaining productive and resilient agricultural systems [18].

2. Chemical and Biological Nature of HSs

2.1. Definition, Composition, and Property

HSs have been a subject of study for centuries, with their role in soil and environmental processes gradually evolving over time. Initially viewed as simple organic residues, the concept of HSs has deepened, reflecting their complex nature and ecological importance. Over the years, scientific advancements have refined their chemical classification

and biological functions (Table 1). Today, HSs derived from the decomposition of plant and animal residues in the environment represent a distinct chemical class of organic compounds. According to the International HS Society (IHSS), HSs are complex, heterogeneous mixtures of polydisperse materials formed through biochemical and chemical processes during the decay and transformation of plant and microbial matter in soils, sediments, and natural waters.

Table 1. Summary of the key historical definitions of HSs, along with the associated references.

Year	Author	Definition	References
1806	Jöns Jakob Berzelius	Described HSs as dark organic matter found in Swedish water sources.	[19]
1932	Selman A. Waksman	Classified HSs into humic acid, fulvic acid, and humin, emphasizing their microbial origin.	[20]
1947	Maria V. Kononova	Defined HSs as products of organic matter decomposition, essential for soil fertility.	[21]
1972	F.J. Stevenson	Characterized HSs as heterogeneous polymers crucial for nutrient cycling and soil structure.	[22]
1989	R.S. Swift	Formalized the definitions of humic acids, fulvic acids, and humin based on solubility, via the IHSS.	[23]
2000s	IHSS 2000s	Defined HSs as heterogeneous mixtures from decomposing plant and microbial matter, important for soil health and ecosystems.	[24]

As significant components of natural organic matter (NOM), HSs are broadly classified into three fractions based on their solubility characteristics:

(1) Humic acids: soluble in alkaline conditions (range pH 8–12) become insoluble at pH 6 and precipitate in acidic environments (pH < 2);
(2) Fulvic acids: soluble in water across all pH values;
(3) Humin: insoluble in water at all pH levels [24].

The solubility characteristics reflect the traditional alkali-extraction methods of isolating these fractions from their original matrices. HSs contain chemically reactive functional groups, such as carboxyl and hydroxyl, contributing to their diverse reactivity and environmental roles. HSs' molecular weight and size vary widely, ranging from a few hundred Daltons (Da) to several hundred kilodaltons (kDa). FAs are generally of a lower molecular weight compared to HAs. The chemical composition of HSs reflects their diverse origins and the chemical processes they undergo during humification. The main components include the following:

- Carbon: Typically makes up 50–60% of the mass, present in both aromatic and aliphatic forms.
- Hydrogen: Constitutes 3–7% of the mass, primarily in aliphatic chains and as part of hydroxyl groups.
- Oxygen: Represents 30–45% of the mass, predominantly found in carboxylic, phenolic, and alcoholic groups.
- Nitrogen: Accounts for 1–5% of the mass, mainly derived from proteins, amino acids, and other nitrogenous organic materials.
- Sulfur and phosphorus: Present in minor quantities, often associated with biomolecules like lignin, nucleic acids, or microbial residues.

The elemental ratios (e.g., H/C, O/C) provide insights into the degree of aromaticity, oxidation, and humification of the material. For instance, HAs tend to have higher aromatic content than FAs, which are more oxidized and hydrophilic [24]. HSs also contain inorganic components that are integral to their structure and function in the soil. These inorganic

components mainly consist of minerals, metals, and other elements that are incorporated into the humic structure through various processes such as adsorption, complexation, and co-precipitation [24].

The metal ions contained in HSs are calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), iron (Fe^{2+}, Fe^{3+}), and aluminum (Al^{3+}); these metals can bind to the functional groups in HSs, like carboxyl and phenolic groups, forming complexes that contribute to the overall structure and behavior of HSs in soils. HSs also can contain trace elements like zinc (Zn), copper (Cu), manganese (Mn), and heavy metals like lead (Pb), cadmium (Cd), and metalloids like arsenic (As) that are chelated by HSs, reducing their bioavailability in the soil and mitigating their potential [25]. Some HSs, particularly those derived from peat and lignite, may also contain small amounts of silica, which is incorporated into their structure [26].

2.2. Structural Characteristics

Although HSs lack a defined chemical structure, they are thought to consist of a matrix of aromatic rings linked by aliphatic chains and functionalized with various oxygen-containing groups. These features give HSs their charge and polarity. Carboxylic and phenolic groups confer to HS negative charges, contributing to cation exchange capacity (CEC) [27]. The interplay of polar and nonpolar groups enables interactions with water and organic pollutants [28]. Advancements in analytical techniques, such as size exclusion chromatography (SEC), have enabled the development of macromolecular structural models for HSs, providing valuable insights into the distribution of molecular sizes, the dynamic nature of HSs in various environments, and how small and heterogeneous humic molecules, with molecular masses ranging from a few hundred to a few thousand Daltons, tend to self-assemble into supramolecular conformations stabilized by weak interactions such as van der Waals forces, π–π stacking, CH–π interactions, and hydrogen bonding [28].

These assemblies contribute to the formation of micelle-like structures capable of solubilizing otherwise insoluble organic compounds, emphasizing the multifunctionality of HSs in environmental systems [29]. In aqueous environments, HSs exhibit a pronounced tendency to aggregate due to physical and chemical crosslinking. These interactions include both intra- and intermolecular forces, driven by the hydrophilic and hydrophobic functional groups attached to their aliphatic–aromatic backbones. As negatively charged entities, HSs accumulate at interfaces, where they act as mediators for organic compound solubilization and play a role in the transport of nutrients and contaminants [30]. However, due to the diversity and dynamic behavior of HSs, techniques such as size exclusion chromatography, light scattering, and viscosity measurements, which are reliable for defining polymer structures, struggle to accurately characterize these molecular assemblies.

Historically, HSs were conceptualized as large, stable macromolecular polymers within soil, often described as key structural components of soil organic matter (SOM). This view positioned HSs as significant, relatively inert molecules, playing a central role in the stabilization and long-term storage of organic carbon in soils. However, in recent years, mounting evidence has shifted the understanding of HSs from this traditional polymeric model to one of dynamic, evolving structures.

Recent studies suggest that soil organic matter should be viewed as a continuum of decomposing organic compounds rather than a fixed assembly of complex polymers. Rather than being singular, stable entities, HS are now understood as highly heterogeneous, consisting of a wide array of molecules that evolve over time through microbial decomposition and chemical processes in the soil.

Modern analytical techniques, such as advanced spectroscopic and chromatographic methods, have provided new insights into the composition and behavior of HSs. These

techniques show that HSs make up only a small fraction of total organic matter (TOM), challenging the earlier assumption that they are the dominant form of organic material in soils. Instead, HSs are now viewed as "supramolecular complexes" or "super mixtures", which are collections of smaller molecules held together by non-covalent forces such as hydrogen bonding, hydrophobic interactions, and van der Waals forces. These associations, rather than persistent covalent bonds, give rise to the molecular masses and properties of humic substances. This new perspective acknowledges that HS do not possess the rigid, defined molecular structure once attributed to them. Instead, they are fluid and dynamic, with properties that change over time and in response to environmental conditions. The molecular makeup of HSs reflects the ongoing transformation of organic matter in the soil, suggesting a far more complex interaction between organic molecules, microorganisms, and soil minerals than previously thought. This alternative view emphasizes the role of HS as part of a broader system of molecular interactions, rather than as isolated, stable macromolecules.

In conclusion, the current understanding of HS reflects a significant shift in how soil organic matter is perceived. Rather than being seen as large, stable polymers, HSs are now recognized as dynamic, non-polymeric complexes whose properties are largely governed by non-covalent interactions. This shift in perspective has important implications for our understanding of soil chemistry, carbon cycling, and the roles of organic matter in soil fertility and ecosystem health (Piccolo et al., 2022; Zhao et al. 2023; Schmidt et al., 2023). Globally, HS production is estimated at approximately 10 petagrams per year, yet their precise chemical structures remain elusive [31]. The formation of HSs is highly dependent on biotic and abiotic factors, including the nature of the parent material, microbial activity, and environmental conditions. This variability underscores the challenge of unambiguously characterizing HSs while highlighting their remarkable versatility.

The unique functional properties of HSs, including their amphiphilic character, molecular size distribution, surface accessibility, and porosity, make them critical components in soil–plant systems. These properties influence the chemical and biochemical behavior of HSs, such as their role in nutrient cycling, pollutant adsorption, and soil structure enhancement. HSs are particularly effective in immobilizing heavy metals and synthetic dyes, such as azo dyes, which are persistent pollutants with harmful ecological and agricultural impacts. Addressing these challenges, HSs serve as natural agents for environmental remediation, demonstrating their importance in both terrestrial and aquatic ecosystems [32].

2.3. Sources and Formation Processes of HSs: A Modern View

The formation of HSs in soil is a complex process involving the chemical and biological transformation of organic matter derived from plant, microbial, and animal residues. The traditional formation view of HSs was based on the microbial and abiotic degradation of organic matter, followed by secondary synthesis processes. Key precursors include lignin, carbohydrates, proteins, and lipids, which undergo oxidative and enzymatic modifications. The humification process was viewed as the condensation of smaller molecules into larger, more stable macromolecules with high resilience to microbial degradation [33]. The formation of HSs occurs through two interrelated pathways. In the first, microorganisms break down complex organic materials such as lignin, cellulose, proteins, and lipids into smaller, simpler molecules such as phenols, carboxylic acids, and amino acids. In the second pathway, these reactive intermediates polymerize through condensation reactions, resulting in the synthesis of larger, more stable macromolecular structures characteristic of HSs [32]. This interconnected process transforms diverse organic inputs into HAs, FAs, and HUs, which contribute to the stability and functionality of SOC. This cycle not only highlights the pivotal role of microorganisms but also underscores the importance of abiotic

factors like oxidation in forming the resilient compounds integral to soil health. The "dead-end" in the microbial degradation of SOM, which refers to stages where decomposition reaches a limit because the organic matter has become too complex or stable for microbes to further degrade, can effectively answer the critical question related to which microbes and which mechanisms can explain the catabolic and/or the anabolic processes in the case of the humus formation. Catabolic and anabolic humus process mechanisms can happen in two ways: Organic compounds, such as highly polymerized lignin or other recalcitrant materials, may reach a point where they are very difficult for microbes to break down. These compounds can become part of the humus fraction without being further degraded. The presence of such "dead-end" materials can contribute to the formation of stable humus. Microbial exhaustion: microbial activity may slow or stop when essential nutrients (like nitrogen or phosphorus) become limited or when the organic material becomes too decomposed. This can lead to a plateau in microbial degradation, where no further breakdown occurs and the SOM remains as part of the humus pool (Schmidt, et al. 2011).

A different approach, which involves initial decomposition followed by synthesis, was further refined by Kononova [34]. It posits that HSs consist of macromolecules formed de novo through the condensation of monomers. According to this theory, FAs are first produced, being of lower molecular weight, and later evolve into higher molecular weight HAs [35]. While the monomer-condensation model provides valuable insights, the degradative theories of HS formation are more widely accepted. These emphasize the oxidative degradation of biopolymers as a critical step in forming humified organic matter (HOM). This perspective is broadly endorsed by both proponents and critics of the humic concept. Supporters highlight the biodegradation of lignin, cutin, suberin, and microbial macromolecules (e.g., melanin) as significant contributors to humification. Such degradation transforms stable plant and microbial biomolecules into simpler intermediates, which are subsequently stabilized through oxidative polymerization and condensation processes. Quinone intermediates such as phenolic and lignin-derived compounds oxidize to quinones, which act as key precursors for humification. These quinones polymerize into larger, more complex structures resistant to further microbial degradation, and the chemical bonds form between different organic molecules, creating a heterogeneous mix of high-molecular-weight compounds [36]. The chemical spontaneous reactions in soils are key processes that lead to the breakdown of macromolecules into smaller units, promote the formation of stable aromatic structures, combine smaller molecules into larger, complex humic macromolecules, interact with metals to stabilize the structure of HSs and impact soil fertility by influencing nutrient availability, result in chemical bonds formed between different organic molecules, and create a heterogeneous mix of high-molecular-weight compounds. The interplay between degradation and synthesis underscores a continuum of humification, where complex macromolecular structures form from simpler precursors under specific environmental and biological conditions. This integrated perspective reconciles various theories, emphasizing the roles of both the degradation of existing biopolymers and the formation of new compounds in shaping the humic fraction of SOC.

Despite differences in the specific details proposed by various research groups regarding the polymerization and formation of HSs in soil, there is a consensus on four experimentally supported principles:

(1) Initial Degradation of Organic Matter: Dead organic matter in soil, predominantly derived from plants and soil microorganisms, undergoes partial degradation. This process breaks down complex molecules into smaller units, such as phenols, phenylpropene derivatives, amino acids, peptides, amino sugars, and simple sugars [37].
(2) Polymerization and Formation of HSs: These smaller molecules subsequently react through polymerization and polycondensation processes. These reactions are

facilitated partly by soil oxidoreductase enzymes or mineral surfaces and partly mediated by microbial activity in the soil. The resulting products are medium- to high-molecular-weight organic compounds, specifically HSs. These humic molecules further associate through supramolecular bonds, forming a dynamic network of interconnected components [38];

(3) Stability and Resistance of HSs to Degradation: these occur mainly due to the
   - high stability and resistance to microbial decomposition, as many humic molecules are inherently stable against microbial enzymes;
   - complexation with inorganic soil components during and after their formation, forming complexes with minerals (e.g., clay) and di- or trivalent cations like Ca^{2+}, Cu^{2+}, Fe^{3+}, and Al^{3+}. These interactions, including sorption to mineral surfaces and cation binding, significantly slow the degradation of organic matter in soil [39].

(4) Structural Models of HSs: The chemical properties and behaviors of HSs allow the development of structural models. These models commonly depict an aromatic core, though variations exist in the degree of aromaticity, molecular weight, and intramolecular bonding. Reviews by researchers such as Stevenson [22] and Schnitzer [40] provided frameworks for understanding these structures, highlighting differences in the spatial organization and chemical complexity of HSs [41].

Research revisited traditional views on humification, formation, and structure, shifting towards a model that emphasizes a continuum of decomposition reactions rather than distinct stages [42]. Despite this shift, evidence continues to support the idea that HSs are distinct molecular products formed under specific environmental conditions. Several key advancements have contributed to this nuanced understanding. Recent research has challenged traditional perspectives on humification and the nature of HSs in soil, shifting towards the "soil organic matter continuum" model [35]. This approach emphasizes a gradual transformation of organic matter rather than the creation of distinct chemical entities like HAs, FAs, and HUs. The continuum model proposes that organic matter is dynamically degraded through biological and chemical processes, forming a spectrum of decomposition products rather than specific, stable compounds, as previously thought. Critics of this model argue that distinct HSs do exist, formed through both biological and chemical reactions, including polymerization and condensation processes. These substances play essential roles in soil, such as enhancing nutrient availability, improving soil structure, and aiding microbial activity. Some researchers also highlight that humic fractions, even if operationally defined, provide valuable insights into environmental processes like nutrient cycling and contaminant binding [43]. A key debate revolves around methodological issues: traditional alkali-based extractions are criticized for potentially creating artificial results that may not represent natural soil conditions. However, proponents argue that such methods still reveal critical functional aspects of HSs in soil ecosystems. This evolving understanding has significant implications for agriculture, environmental management, and climate studies, as it refines how we conceptualize soil carbon dynamics and organic matter interactions [44]. The role of soil fauna, including organisms like insects and earthworms, facilitated humic formation through enzymatic and alkaline conditions in their digestive systems; synthesis technologies such as hydrothermal carbonization provide insights into natural processes and enable applications in soil improvement and pollution management. The integration of technologies, including hydrothermal carbonization, has enabled the synthesis of artificial HSs. These techniques, replicating natural formation processes, offer practical applications in soil improvement and pollution management. Innovation such as nuclear magnetic resonance (NMR) spectroscopy and Fourier-transform infrared (FTIR) spectroscopy have revealed detailed structural information about HSs, enhancing our

understanding of their formation pathways and functional properties [45]. This nuanced view integrated traditional and modern theories, highlighting HSs as vital components of SOC with critical roles in soil fertility, nutrient cycling, and environmental health. These advancements integrate traditional theories with modern insights, underscoring HSs as essential components of SOC. They play critical roles in improving soil fertility, supporting nutrient cycling, and maintaining environmental health. The time HSs need to remain in soil to benefit soil health and plant growth can vary depending on several factors, such as soil type, climate, and the specific soil management practices in place. However, the benefits of HSs typically become noticeable over the course of several months to a few years [46–48].

Short-term benefits (months to a year): HSs begin to improve soil structure relatively quickly by promoting the aggregation of soil particles. This leads to improved soil aeration, water infiltration, and root development within a few months. They also enhance nutrient availability by increasing the CEC, allowing plants to access more nutrients.

Medium-term benefits (1 to 3 years):

As HSs continue to stabilize in the soil, their impact on soil health becomes more pronounced. Soil microbial activity increases, enhancing organic matter decomposition and nutrient cycling. The improved soil structure supports better root growth, contributing to increased plant vigor and crop yield.

Long-term benefits (3 years and beyond):

Over the long term, HSs contribute to sustained soil fertility by enhancing the soil's ability to store nutrients and water. The stabilization of organic matter helps in reducing soil erosion, improving carbon sequestration, and maintaining soil health over many years.

In summary, while HSs can start showing benefits in soil health and plant growth within months, their full impact, especially in terms of improving soil fertility and long-term plant productivity, typically takes a few years to manifest. The key is ensuring that HSs remain in the soil long enough to facilitate these lasting improvements. Additionally, the frequency of HS application is important and should be tailored to the specific needs of the soil, crop, and farming system to maximize benefits in terms of soil health, fertility, and crop productivity: For general soil health, once a year (in spring or fall). For degraded or sandy soils, twice a year or more, depending on the degradation level. For high-intensity farming or crops with high nutrient demand, two to three times a year. For organic farming or integrated soil management, annual or bi-annual application, possibly in combination with other organic amendments [47,48]. Recently, HSs have gained widespread attention as effective soil amendments due to their ability to enhance soil fertility, structure, and overall health. Notably, HSs have been shown to stabilize organic carbon in soils over the long term, offering a sustainable solution for improving soil carbon storage [49]. Furthermore, ongoing studies are focused on the integration of HSs into soil management practices, including their use in organic farming, regenerative agriculture, and greenhouse farming, where they can offer a low-cost, environmentally friendly approach to soil fertility management.

3. The Multifaceted Biological Functions of HSs

HAs serve as silent architects of life, influencing biological systems in ways that resonate far beyond their immediate presence. Emerging research reveals that these enigmatic organic compounds play a pivotal role in regulating processes at the intersection of biology and ecology, impacting everything from microbial dynamics to plant health and from nutrient cycling to ecosystem resilience. Their versatility as mediators in biochemical interactions highlights their profound influence on both individual organisms and complex ecological networks [50].

The biological functions of HSs are multifaceted and interwoven, manifesting as growth enhancement in plants, the modulation of microbial populations, and even protective effects against environmental stressors. These interactions hold transformative potential for addressing some of the most pressing environmental challenges, including soil degradation, water contamination, and the global decline in biodiversity. By mediating key processes such as nutrient availability, oxidative stress reduction, and pollutant binding, HSs emerge as critical players in promoting sustainable ecological equilibrium [51].

Knowing the dynamic interplay between HSs and biological systems and illuminating their direct and cascading effects on the environment are nowadays more and more important. Through a lens of innovation and sustainability, we explore how understanding and leveraging the unique functionalities of HSs can offer new pathways for environmental restoration, agricultural productivity, and ecosystem management. The potential of HSs to act as both mitigators of environmental stress and enhancers of biological resilience underscores their significance as a bridge between natural systems and human efforts to mitigate the pressure on the environment [52].

3.1. Soil Property Improvement

HSs positively influence soil's physical, chemical, and biological properties, thereby supporting plant growth. Although the role of HSs as growth stimulators is not a novel discovery, they have gained attention due to their effectiveness in small amounts, offering multiple benefits for plant development [52]. Among their most significant bio-stimulant effects are enhancing root systems by improving cation exchange capacity, water retention, and phosphorus availability and activating plasma membrane H^+-ATPases. They also play a role in stress tolerance [52–54]. These effects arise from interactions among SOC, microbial communities, and plant roots. These interactions contribute to improved soil structure, increased nutrient and water availability, and enhanced microbial activity, all of which help plants better withstand environmental stresses such as drought, salinity, and temperature extremes. HSs improve soil water retention and nutrient availability, reducing the negative impacts of drought stress and promoting better root development under water-limited conditions. They also stimulate the growth of beneficial soil microbes that produce growth-promoting substances, suppress pathogens, and improve the soil's resilience to biotic stresses. Moreover, HSs enhance the plant's antioxidant defense mechanisms by increasing the synthesis of stress-related compounds like phenolic acids, flavonoids, and other secondary metabolites, which help the plant manage oxidative stress induced by environmental factors. Additionally, HSs have been shown to improve soil microbial diversity, leading to a healthier soil ecosystem that supports plant growth and resilience to stress [55]. Additionally, HSs impact primary plant metabolism, enzymatic activities tied to cellular functions, and secondary metabolism. They also contribute to soil structure and aggregation, pH buffering, nutrient bioavailability, water retention, and reducing aluminum or heavy metal toxicity. However, optimizing their application to maximize crop yield and growth remains essential. Variability in HS composition, sources, and application methods creates uncertainties and diverse plant responses. Factors such as the humic source, environmental conditions, target plant species, dosage, and method of application significantly influence their effectiveness [56]. Moreover, HSs influence secondary metabolite production, aiding in stress resistance and disease mitigation [12]. They enhance enzymatic activity linked to stress responses [57] and modulate microbial communities within and beyond the rhizosphere [58]. This modulation improves processes like pathogen defense, nutrient mineralization, and solubilization [52]. Studies conducted in Egypt and in Turkey showed their role in managing plant diseases, affecting crops such as cucumber and tomato [59,60]. The authors tested three concentrations ranging from

50 to 100 and 150 ppm for each HS, HA, and FA for their efficacy in reducing pathogen growth. In vitro, FAs extracted from the green compost of plant residues and biogas manure significantly reduced pathogen growth at a concentration of 150 ppm compared to the control. FAs extracted from plant residues at concentrations of 150 ppm reduced disease severity significantly (84.7%) in relation to the other treatments. A significant increase in the total number of microorganisms in soil by HAs extracted from biogas manure with respect to other treatments have been demonstrated. All applications significantly improved the growth parameters of cucumber plants in the presence of natural inoculum, with the effects of HA treatments being more pronounced. Pathogen suppression mechanisms include microbiotas, the colonization of pathogen propagules, antibiosis, substrate competition, and induced systemic resistance [61].

3.2. Soil Functionality and Microbial Dynamics

HSs are distinguished by their complex and reactive chemical composition, which equips them to engage in a range of critical ecological processes. Their abundance of functional groups, such as carboxyl, phenolic, and quinone moieties, allows them to interact with diverse environmental components. These interactions underpin key processes including nutrient cycling, pollutant detoxification, and the stabilization of organic matter within ecosystems [62].

HSs play a pivotal role in promoting biodiversity within agricultural ecosystems by enhancing both soil microbial diversity and plant diversity. The complex molecular structure of HSs provides an abundant source of carbon and energy for beneficial soil microorganisms such as bacteria, fungi, and actinomycetes [63]. This microbial diversity is essential for nutrient cycling, disease suppression, and the overall health of the soil ecosystem [12]. Furthermore, HSs' role as signaling agents and growth promoters influences plant–microbe interactions and the establishment of symbiotic relationships, such as mycorrhizal associations, which are vital for ecosystem diversity and productivity. Recent evidence demonstrated that different molecular fractions of HSs, based on their size and composition, affect plant resilience and response mechanisms differently under stress conditions. This contributes to maintaining functional diversity in agricultural systems [64]. Recent studies showed that soils enriched with HSs exhibit increased populations of plant growth-promoting rhizobacteria and mycorrhizal fungi, which enhanced nutrient availability and plant resilience to stress [7,65]. These microbes form symbiotic relationships with plants, improving nitrogen fixation, phosphorus solubilization, and resistance to pathogens [11].

In addition to supporting microbial life, HSs improve soil structure and nutrient availability, which fosters plant diversity in agricultural systems [66]. This diversity, in turn, supports a wider range of pollinators, insects, and wildlife, enhancing ecosystem stability and resilience. The application of HSs has been shown to improve the productivity of cover crops and polyculture systems, which are critical for sustainable and regenerative agriculture [15].

Humic products (HPs) are widely applied as soil amendments to enhance plant nutrient uptake and improve agricultural productivity. However, recent research has raised questions about their broader effects on soil biology and microbial dynamics. The interaction of HPs with soil microorganisms and biochemical processes is highly complex, involving both beneficial and detrimental responses. These interactions vary depending on the type, dose, and frequency of HP application.

HPs have the ability to influence specific microbial populations, such as *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes*, while also impacting enzyme activities, including laccase, peroxidase, and dehydrogenase [67]. Studies indicate that HPs can modulate microbial respiration, particularly reducing CO_2 emissions by promoting anaerobic pathways like CH_4 production under certain conditions [68]. This effect is attributed to the redox-active functional groups in HPs, which can act as electron donors and alter microbial electron flow, thus modifying both CH_4 and CO_2 emissions [69].

Moreover, HPs can have diverse effects on the soil ecosystem by influencing the microbial biomass and enzyme-mediated nutrient cycling. For instance, HPs have been implicated in influencing soil enzyme activity, which plays a central role in nutrient cycling. For instance, catalase and urease enzyme activities have been linked to improved N-cycling and overall soil fertility when HPs are applied appropriately [67].

On the other hand, the excessive application of HPs can negatively affect soil biodiversity and stability, with some studies noting reduced microbial diversity and stability at higher doses [70]. This suggests that the concentration and mode of HP application are critical for maximizing their ecological benefits while minimizing adverse effects. HSs derived from organic materials play a crucial role in stimulating microbial activity in soils, as evidenced by various studies highlighting their positive impact on soil health and fertility.

Li et al. [71] demonstrated that HSs from lignite and peat enhanced microbial diversity and activity, promoting the breakdown of organic matter and improving nutrient cycling in soil. The positive effects of HSs on soil microorganisms are attributed to their ability to serve as both a carbon source for microbes and a stimulator of microbial enzymatic activity, thereby improving soil health and nutrient availability [71]. Furthermore, Lumactud et al. [48] reported that the use of HSs led to increased microbial populations and enhanced nitrogen fixation, contributing to higher crop yields and reduced dependency on mineral fertilizers. Da Silva et al. [72] evidenced the positive effects of the inoculation of beneficial bacteria combined with the application of HSs on promoting the growth of non-leguminous plants and how this combination increased the bacterial colonization of plant hosts, showing the potential for the combined use of HSs and plant growth-promoting bacteria (PGPB) as agricultural inputs in different crops. Numerous investigations indicated that incorporating HPs into soil significantly influenced bacterial community composition and abundance, with smaller effects observed on actinomycetes, soil fungi, and microalgae populations [73–75]. Additionally, studies employing the most probable number (MPN) method revealed that HP amendments supported large populations of nitrate-reducing bacteria. In a long-term field study involving peanuts treated with HAs and inorganic fertilizers, alterations in soil enzyme activities were found to significantly impact the structure of the soil microbial community. The treatment increases the beneficial bacterial populations while reducing the abundance of harmful bacteria [76]. Additionally, the application of HAs led to an enrichment of plant-beneficial taxonomic groups, including bacterial genera within Firmicutes and fungal groups such as *Basidiomycota* and *Mortierellomycota*, which are known to enhance plant health and soil quality [77]. Variations in the physicochemical properties of HPs, experimental designs, and prevailing biotic and abiotic factors significantly influence their impact on soil microbial structure and composition. These factors determine how HPs interact with soil systems, including their ability to modulate microbial communities, enhance nutrient availability, and support plant growth.

All the multifaceted biological functions of HSs described in Section 3 are summarized in Table 2.

Table 2. Summary of multifaceted biological functions of HSs.

Key Findings	Impacts of HSs	References
Soil Property and Functionality Improvement	HSs enhance soil physical, chemical, and biological properties, improving root systems, nutrient availability, water retention, and stress tolerance. They help plants withstand environmental stresses like drought and temperature extremes.	[52–55]
Soil Microbial Activity and Disease Control	HSs stimulate beneficial soil microbes, promote growth, suppress pathogens, and improve soil resilience. They help reduce pathogen growth and disease severity in crops like cucumber and tomato. They also improve microbial diversity, nutrient cycling, and soil health.	[59–61,63]
Nutrient Cycling, Pollutant Detoxification, and Organic Matter Stabilization	HSs facilitate nutrient cycling, pollutant detoxification, and organic matter stabilization within ecosystems, supporting overall soil health.	[62]
Microbial Respiration and Enzyme Activity	HPs influence microbial respiration, reduce CO_2 emissions, and promote anaerobic processes. They also improve enzyme activity (e.g., catalase, urease), enhancing soil fertility and N-cycling.	[67–69]
Microbial Diversity, Stability, and HS Applications	Excessive HP application may reduce microbial diversity and soil stability, but optimal applications can enhance microbial community composition, including beneficial bacteria and fungi, improving plant health and soil quality. HSs, especially those derived from organic materials, stimulate microbial activity and increase microbial diversity, supporting nutrient cycling and soil fertility.	[70,71,73–77]
Combination of HSs and PGPR	HSs combined with PGPR increase bacterial colonization and crop yields and reduces the need for mineral fertilizers.	[72]

4. The Effects of HSs on Plant Growth and Physiology

4.1. Root Responses to HSs

HSs are emerging as natural growth enhancers, reshaping our understanding of plant physiology and development. By acting as bioactive catalysts, HSs influence key processes such as nutrient uptake, root architecture, and stress resilience, fostering healthier and more robust plants. Beyond conventional fertilizers, these organic compounds engage directly with cellular signaling pathways, modulate enzymatic activity, and improve soil–plant interactions, offering a sustainable and eco-friendly approach to boosting agricultural productivity [78]. Exploring the multifaceted effects of HSs on plant growth reveals a transformative tool for addressing the global challenges of food security and environmental sustainability.

The HSs emerged as vital players in the regulation of plant root growth, development, and adaptation to environmental stressors. Recent studies suggested that HSs act as signaling molecules, influencing root architecture, nutrient uptake, and stress resistance mechanisms [79]. Humic substances may also influence auxin signaling pathways. Auxin regulates gene expression by binding to receptors in plant cells, triggering a cascade of responses that control growth and development. Humic substances, through their interaction with plant root membranes and cell walls, may alter the activity of auxin receptors or affect the transport of auxins to specific target sites within the plant. By modulating these signaling pathways, humic substances can enhance or fine-tune the plant's responses to environmental stimuli, such as light, gravity, and stress. A study of Muscolo et al. [66] and Nardi et al. [12] demonstrated that HSs can induce physiological effects typical of auxin (AUX), and AUX was detected in their structure, as discussed in earlier reviews. The AUX-like effect represents one of the most well-documented phytohormonal responses triggered by HSs and has been investigated for over 50 years [56,75]. This effect is evident in morphological changes such as root elongation and the initiation of lateral root emergence, which are well-established responses to HA applications [73]. The AUX-like effects are pri-

marily linked to the activation of the plasma membrane (PM)-bound H^+-ATPase enzymes, facilitated by small bioactive compounds with auxinic properties found in HAs, such as indole-3-acetic acid (IAA) [80]. HSs exhibit AUX-like activity, significantly influencing root growth and physiology by mimicking the action of indole-3-acetic acid (IAA), a primary AUX in plants. Research by Muscolo et al. [81] and Russel et al. [82] demonstrated that HSs modulate plant responses by stimulating plasma membrane (PM) H^+-ATPase activity, which promotes H^+ efflux and acidifies the apoplast. This results in cell wall loosening and enhanced cell expansion. Additionally, HSs influence secondary transporters, such as ZmNrt2.1 (a nitrate transporter), linked to their AUX-like effects. Further studies highlight HSs' role in signal transduction pathways mediated by enzymes like phospholipase A2 and protein kinase C, mirroring IAA's physiological responses in plants.

Data from a range of plant species indicated that specific inhibitors of AUX (2,3,5-triiodobenzoic acid and 4-chlorophenoxy-isobutyric acid) and anti-IAA antibodies can inhibit HS biological activity [83].

The interaction between auxins and HSs enhance the plant's ability to cope with environmental challenges by optimizing stress-related growth processes, such as the development of deeper root systems or the regulation of stomatal closure to conserve water, helping to regulate key developmental processes beyond root growth. HSs modulate this process by influencing auxin transport and distribution within the plant, potentially leading to more balanced shoot growth and better branching patterns. This could improve overall plant structure, supporting greater leaf area and better photosynthetic efficiency.

Numerous studies showed that the hormone-like activity is not only linked to HSs but also to composts [84] and raw materials [85]. Scaglia et al. [86] found that the dissolved organic matter from pig slurry digestate had AUX-like activity assigned to the presence of aromatic amino acids coming from the anaerobic digestion, whereas no gibberellin-like activity was detected. Later, Scaglia et al. [87] extracted substances with hormone-like activity from raw materials and corresponding vermicompost, which were well identified by using GC/MS. Carboxylic acids and amino acids were classified as molecules involved in the IAA-like activity [88].

A study evidenced that root exudates, such as phyto-siderophores, alter the structural properties and molecular conformation of HSs, creating a dynamic interaction each other's [89]. Conversely, HSs regulate the release of specific exudates, which becomes particularly relevant under conditions of stress, such as iron (Fe) deficiency [12,74]. These small molecules interact with specific cell receptors to initiate intracellular signaling pathways. This leads to the extrusion of H^+ ions via P-type ATPase enzymes, which acidifies the apoplastic space and triggers pH-sensitive enzymes. These enzymes weaken the cell wall structure, thereby promoting cell expansion by facilitating increased turgor pressure, in coordination with vacuolar-type H^+-ATPase anchored at the tonoplast membrane [90].

Moreover, the morphological effects induced by HSs closely mimic the responses triggered by IAA, as evidenced by experimental treatments with IAA inhibitors, which block root development pathways in maize treated with HAs [74].

New perspectives highlighted the role of HSs as active regulators of root phenotypic plasticity. A different study showed that HSs influenced lateral root development, root elongation, and the formation of symbiotic relationships (e.g., mycorrhizal colonization), thereby enhancing a plant's ability to access resources under varying environmental conditions, including salinity, drought, and heavy metal toxicity, by modulating oxidative stress pathways and nutrient availability [89,90]. Garnica et al. [91] explored the effects of HAs on root mechanisms in plants under iron (Fe) deficiency, with a particular focus on graminaceous species such as wheat. Their investigation was motivated by the limited scope of previous research, most of which concentrated on dicotyledonous plants and established

the role of HAs in enhancing Fe solubility and bioavailability [92]. Their study specifically assessed whether a peat-extracted HA could improve Fe nutrition in both Fe-deficient and Fe-sufficient wheat. Results demonstrated that HA application improved the physiological condition of Fe-deficient plants by increasing the Fe-active pool in their leaves. This effect was likely due to the mobilization of Fe complexes via interactions between HAs and phytosiderophores released into the nutrient solution [93]. Moreover, the translocation of Fe from roots to shoots appeared to be facilitated by the phytohormone trans-Zeatin Riboside (tZR), whose concentration was notably elevated in the leaves of Fe-deficient plants treated with HAs. These findings underscored the role of HAs not only in improving Fe bioavailability, but also in enhancing hormonal signaling pathways that contribute to improved nutrient transport and plant resilience under Fe-limiting conditions [91,92]. This evolving understanding positions HSs as integral modulators of root systems rather than merely passive byproducts of organic matter decomposition. In conclusion, recent insights into root responses to HSs reveal their pivotal role in modulating root architecture and function. HSs stimulate root elongation, branching, and surface area expansion, enhancing the plant's ability to access water and nutrients. These effects are mediated through complex signaling pathways, including hormone-like activities and interactions with nutrient availability (Table 3). Such advances in understanding underscore the potential of HSs as natural agents for optimizing root development and improving plant resilience in sustainable agricultural systems.

Table 3. Summary of key studies on root responses to HSs.

Role of HSs	Key Insights	References
Auxin-like Effects on Root Growth	HSs exhibit AUX-like activity, promoting root elongation, lateral root formation, and the initiation of root development. This effect is linked to the activation of plasma membrane H^+-ATPase enzymes, which acidify the apoplast, loosen the cell wall, and enhance root cell expansion. Additionally, small bioactive compounds with auxinic properties found in HSs, such as indole-3-acetic acid (IAA), play a key role.	[12,81,82]
Modulation of Oxidative Stress and Nutrient Availability	HSs influence lateral root development, root elongation, and symbiotic relationships (e.g., mycorrhizal colonization), which improve nutrient access and plant resilience to stress, including salinity, drought, and heavy metal toxicity. These processes are often regulated through hormonal pathways and interactions with environmental stress factors.	[86,87]
Regulation of Root Exudates and Stress Response	HSs regulate root exudates under stress, particularly during Fe deficiency, by influencing the release of specific exudates. These interactions initiate intracellular signaling pathways, promoting root development and resilience under nutrient-limited conditions.	[12–92]
Hormonal Regulation in Iron Deficiency	HSs enhance Fe solubility and bioavailability, improving Fe nutrition in Fedeficient plants. This effect is associated with hormonal signaling pathways, including the translocation of Fe via phytohormones like trans-Zeatin Riboside (tZR).	[92,93]

4.2. HSs: Catalysts for Plant Physiological Adaptation

Key findings by Nardi et al. [12] underscored the role of specific functional groups within HSs in triggering both localized and systemic physiological responses in plants. These responses are mediated through intricate hormone-like signaling pathways. The biological activity of HSs is highly dependent on several factors, including their concentration, origin, molecular size, hydrophobicity, aromaticity, and the spatial arrangement of their hydrophilic and hydrophobic domains. It was brought to light that the molecular size of HSs plays a critical role in determining their mode of action [12]. Low-molecular-weight HSs can penetrate root cells and directly initiate intracellular signaling cascades, whereas

high-molecular-weight HSs interact with external cell receptors to trigger downstream molecular responses. The primary targets of HSs in plants include nutrient transporters, plasma membrane H^+-ATPases, hormone signaling pathways, genes and enzymes related to nitrogen assimilation, and mechanisms governing cell division and development. Additionally, HS-induced responses extend beyond direct enzymatic activation. Genes linked to secondary nutrient transport mechanisms—such as ZmNr t2.1, a nitrate transporter—are considered key candidates for contributing to the AUX-like effects observed in plants under HA treatment [94]. Another study further demonstrated that stomatal responses, such as the opening of stomatal pores in pea epidermal tissue, were comparable to those induced by IAA treatments. This response was linked to phospholipase A2 (PLA2) and protein kinase C-like (PKC) activity, both of which are integral components of the intracellular signal transduction pathways activated by IAA in response to environmental and hormonal cues [12]. These findings highlight the multifaceted signaling mechanisms activated by HSs, mediated by both enzymatic activity and hormonal pathways. Recent work by Nardi et al. [95] evidenced that the effects of HSs were largely influenced by their molecular structure and that changes in their molecular conformation and interactions with plant roots led to diverse biological outcomes.

Pantoja-Guerra et al. [96] analyzed how the auxin-like effect in the soil–plant system modulates the plant growth-promoting activity (PGB) of *Lysinibacillus pinottii* sp. nov. PB211. The effect of HOM soil was simulated with HAs coming from vermicompost; the spectral configuration (CP/TOSS and FTIR) and coleoptile elongation test indicated its bioactivity. Cucumber exhibited a PGP effect of PB211 and HAs at a lower concentration compared to corn. This disparity agrees with the differential sensitivity to auxins of both plant models. Monocot plants, such as corn, generally exhibit greater resistance to exogenous auxin activity compared to eudicot plants, such as cucumber. The presence of HAs in the growth substrate (sand) was found to modulate the plant growth-promoting rhizobacteria (PGPR) activity of PB211, resulting in a loss of PGPR activity. Wang et al. [97], conducted a detailed study to evaluate the effects of two distinct HSs, Aojia humic acid (AHA) and Shandong humic acid (SHA), on maize (*Zea mays*) by employing foliar spraying. These HSs were previously characterized and selected based on their molecular compositions. Plant samples were collected ten days after spraying (62 days post-germination) to assess their impacts on photosynthesis, dry matter accumulation, and carbon and nitrogen metabolism, as well as broader metabolic pathways. Using Electrospray Ionization–Overpressured Layer Chromatography–Mass Spectrometry (ESI-OPLC-MS) technology, the study identified 510 small molecules with significant compositional differences between AHA and SHA. These compositional differences were reflected in the distinct biological effects on maize growth and metabolism, with AHA eliciting a more pronounced stimulatory effect compared to SHA. HSs triggered specific changes in plant metabolic pathways, enhancing carbohydrate availability, boosting energy production and metabolic flux, potentially influencing plant secondary metabolism and stress responses, and modulating nutrient and metabolite transport across cellular membranes. Rathor et al. [85] showed that the bio-stimulatory effects of HAs arise primarily from their ability to influence innate auxin and cytokinin biosynthesis pathways in plants. The authors performed analyses on the gene expression targeting pathways involved in auxin and cytokinin biosynthesis on wheat seedlings. Additionally, transgenic lines of *Arabidopsis thaliana* carrying auxin-responsive DR5 and cytokinin-responsive ARR5 promoters fused with a β-glucuronidase (GUS) reporter were employed to evaluate GUS expression in seedlings treated with HAs. The findings revealed that HA application enhanced both shoot and root growth in wheat seedlings. Genes associated with auxin biosynthesis, such as Tryptophan Aminotransferase of *Arabidopsis* and Gretchen Hagen 3.2, as well as the cytokinin biosynthesis gene Lonely

Guy3, exhibited upregulated expression in treated plants compared to controls. Furthermore, GUS expression analysis indicated that HA bioactive components triggered auxin- and cytokinin-like activities, supporting their role in modulating hormonal pathways. This research is a pioneering report utilizing ARR5:GUS transgenic lines to confirm the biostimulant properties of HAs and their potential to stimulate endogenous hormone-like responses in plants [97].

In short, the last evidence underscores that HSs influence plant development through a combination of direct and indirect mechanisms, mediated by their molecular structure, origin, and physicochemical properties. Key findings reveal that HSs enhance plant growth by modulating central metabolic pathways, nutrient assimilation, and stress resistance. For instance, they reorganize critical pathways such as starch and sucrose metabolism, the TCA cycle, and lipid biosynthesis, depending on their molecular characteristics. HSs interact with plant cell surfaces and root exudates to create dynamic molecular interactions that alter their conformation and bioactivity. This interaction enhances their efficacy in promoting root and shoot growth, increasing nutrient availability, and improving overall plant resilience. Compost-derived HAs, with higher polarity and hydrophilicity, are particularly effective, highlighting the importance of source-specific applications for maximizing benefits.

While the beneficial effects of HSs are well established, this field still requires further exploration to unravel the precise structure–function relationships and conformational dynamics driving these responses. Continued research is essential to optimize the use of HSs as sustainable tools for improving crop productivity, soil health, and stress resistance, making them indispensable in the transition to environmentally friendly agricultural practices.

4.3. HSs: Natural Stress Resilience Boosters for Plants

The HSs have garnered attention for their role in mitigating abiotic stress in plants, such as drought, salinity, heavy metal toxicity, and temperature extremes. HSs aid in stress mitigation through mechanisms like enhancing root growth, improving ion uptake, stabilizing cell membranes, and activating antioxidant systems to combat oxidative stress. Their ability to modulate hormonal balance and signaling pathways further supports plant resilience under environmental challenges, making HSs a key natural ally in sustainable agriculture [17].

Recently, Yildirim et al. [98] evidenced that HA + FA successfully mitigated the negative impacts of Cd pollution, in garden cress, by enhanced N, P, K, Ca, Mg, Fe, Mn, Cu, Mn, Zn, and B. In addition, HA + FA applications at a certain concentration successfully reduced Cd uptake by 95% and Cl uptake by 80% by decreasing H_2O_2, MDA, proline, and sucrose values. Muscolo et al. [99] isolated thirteen different HAs and FAs from diverse sources, including soils, composts, peat, leonardite, and lignite and characterized their carbon component distribution using $^{13}C^{13}C$-CPMAS NMR spectroscopy. The study utilized callus cultures of *Pinus laricio* as a model plant system to evaluate the effects of these humic extracts on growth and various biochemical parameters. These included carbohydrate and phenol content, activities of nitrogen metabolism-related enzymes (invertase, glutamine synthetase, glutamate synthase, phosphoenolpyruvate carboxylase, and malate dehydrogenase), and stress-related enzymes like catalase. The findings evidenced that humic materials enhanced callus growth, as expected, with the greatest activity exerted by the most polar HAs coming from composts. The work confirmed that HSs of various origins could act as sustainable biostimulants of plant growth and as stressor protectors by increasing phenols and antioxidant enzymes. The mechanisms underlying stress mitigation in plants encompass a diverse array of processes, including the activation of signaling pathways, induction of stress-responsive genes, accumulation of osmo-protectants and

antioxidant molecules, enhancement of antioxidative metabolic activity, regulation of ion homeostasis, facilitation of membrane transport, and modulation of hormonal balance. This multifaceted response raises a compelling question: how does a complex interplay of molecules orchestrate such a wide spectrum of physiological and biochemical adaptations, enabling plants to thrive under adverse conditions? In agreement with the findings of Canellas and co-worker [17], HSs contain both pro-oxidant components, such as quinone moieties, and antioxidant groups, including phenolic hydroxyls, within their complex chemical structures [99]. This duality enables HSs to participate actively in redox reactions. The growth-promoting effects of HSs on plants may, therefore, be partly attributed to redox dynamics, influenced by the balance between pro-oxidant and antioxidant groups within their structure. Pro-oxidant moieties in HSs can contribute to reactive oxygen species (ROS) generation, triggering apoplastic oxidative bursts that play a role in signaling pathways. Conversely, antioxidant moieties, particularly polyphenolics, help regulate these oxidative bursts, preventing excessive damage. Additionally, plants respond to the oxidative environment by upregulating their intrinsic antioxidant defense systems. This coordinated interplay restores redox homeostasis, protects critical cellular components like membranes, proteins, and nucleic acids, and ultimately enhances stress tolerance, growth, and productivity. This nuanced redox-based mechanism highlights the multifaceted role of HSs in plant development and resilience.

These findings highlight the crucial role of humic substances (HSs) in promoting plant growth under optimal conditions through hormonal and metabolic effects, as well as enhancing resilience under abiotic stress by regulating redox balance and enzyme activation, summarized in Table 4.

Table 4. Summary of effect on plant growth and stress resilience of HSs.

Effect of HSs	Key Studies and Findings	References
	Optimal Conditions (Plant Growth Promotion)	
Hormonal-like effects and physiological adaptations	HSs trigger hormonal-like responses, such as auxin and cytokinin regulation, enhancing root and shoot growth. Low-molecular-weight HSs penetrate cells and initiate intracellular signals; high-molecular-weight HSs trigger cell surface receptors.	[12,88]
Molecular structure-dependent effects	Different HS sources, such as those from vermicompost, modulate plant growth-promoting bacteria (PGPB) activity in a concentration-dependent manner.	[96]
Molecular metabolism modulation	HSs impact plant metabolism, enhancing carbohydrate availability and energy production and influencing secondary metabolism and stress responses.	[97]
	Stressful Conditions (Abiotic Stress Mitigation)	
Drought, salinity, heavy metals, oxidative stress	HSs mitigate stress by enhancing root growth, ion uptake, stabilizing membranes, and activating antioxidant systems. Redox dynamics regulate oxidative bursts, balancing pro-oxidant and antioxidant activities.	[17,98,99]
Heavy metal toxicity	HSs reduce cadmium (Cd) uptake by up to 95%, Cl uptake by 80%, and protect plants from oxidative damage (reduced H_2O_2, MDA, proline, sucrose).	[98]
Redox-based stress tolerance	The antioxidant and pro-oxidant components of HSs participate in redox reactions, enhancing plant stress resilience by improving oxidative stress tolerance.	[99]
Stress-related enzyme activation	HSs increase the activity of enzymes related to nitrogen metabolism (e.g., glutamine synthetase) and stress-related enzymes (e.g., catalase).	[99]

5. Building a Greener Future: HSs in Sustainable Farming

HSs are emerging as potent biostimulants with significant implications for sustainable agriculture, owing to their multifaceted effects on plant growth and physiology. According to De Moura et al. [100], HSs demonstrated a notable ability to enhance plant metabolism when applied via foliage. The authors examined the specific traits of foliar applications and HSs across various plant categories, including vegetables, grasses, legumes, fruits, oilseeds, medicinal plants, and ornamentals. Across all plant groups, the foliar application of HSs consistently boosted parameters such as biomass production, plant height, photosynthetic

pigment levels, and overall agricultural yield. Moreover, foliar-applied HSs enhanced plant resilience against stress by increasing the activity of key stress-related enzymes like peroxidase, catalase, and phenylalanine ammonium lyase (PAL). The quality of fruits treated with HSs also improved, with significant increases in sugar content, as well as higher levels of oil, protein, and fiber. For instance, research by Jan A.J et al. [101] demonstrated that the foliar application of ha improves growth and yield of chili (*Capsicum annum* L.), especially for the foliar application of HAs at the rate of 50 gL^{-1} [102]. Two recent studies on potato (*Solanum tuberosum* L.) found that the foliar application of HSs increased tuber yield [102,103]. Other researchers, on the other hand, conducted a study on the foliar application of HAs on a species of a featured in Table 1 (*Vitis Vinifera* L.) at concentrations of 5 and 20 mg L^{-1}, finding that both concentrations resulted in an increase in crop yield, berry size, chlorophyll content, N in leaves and petioles, and % TSS, as well as a reduction in fruit titratable acidity and a delay in chlorophyll degradation [104].

These improvements are attributed to the ability of HSs to enhance nutrient uptake, stimulate enzymatic activity, and improve soil health, all of which contribute to better fruit quality and nutritional content. These findings underscore the potential of HSs as an effective tool for improving the quality and marketability of fruits in agricultural systems [105]. These evidences highlighted the importance of conducting experiments across diverse plant species, environmental conditions, and sources of HSs to optimize application practices. The findings confirmed that HSs are an environmentally friendly, cost-effective technology with the potential to benefit small-scale and family farmers by improving productivity and sustainability in agriculture.

Chen et al. [106] showed that HSs play a multifaceted role in enhancing plant resilience and growth through several interconnected mechanisms. Notably, these substances aid in osmotic regulation by helping to maintain the plant's water balance and mitigating the impacts of drought stress, with important implications for sustainable agriculture due to the water savings that result from the use of HSs for crop cultivation. Ampong et al. [107] evaluated the significant impact of HAs on various aspects of crop growth and development, including plant hormone production, nutrient uptake and assimilation, yield enhancement, and protein synthesis. The effects of HAs on both soil properties and crop performance were strongly influenced by several factors, such as the type of HA, and the application identified several factors that affect HA performance in crops and soils; the most influential of them is the HA source. The HA chemical and molecular structure, solubility, and other factors such as application rate, soil, and crop type also affect HA effects on crop performance [105]. Despite the promising benefits, this review identifies critical gaps in the current understanding of HA applications. For instance, there is a lack of field studies examining the effects of HAs across diverse crop rotation systems, forms of nitrogen fertilizers, geographic locations, and varying climatic conditions. Additionally, the influence of HA and their water- and alkaline-soluble fractions, remains largely unexplored in field experiments, particularly in relation to crop yield, quality, and soil health.

Recent research highlights the benefits of integrating HSs with organic amendments for sustainable soil management. This combination has been shown to improve soil fertility, enhance nutrient availability, and foster microbial activity. For instance, studies reveal that HSs combined with organic inputs like compost or biochar significantly improve soil organic matter stability and enhance nutrient cycling, leading to better plant growth and yield [108,109]. The synergy between HSs and organic amendments facilitates the formation of stable soil aggregates, improving water retention and reducing erosion. Moreover, these mixtures enhance the bioavailability of essential nutrients like nitrogen and phosphorus while mitigating the leaching of harmful substances, contributing to environmental sustainability [110]. Advanced techniques such as two-dimensional spec-

troscopy (2D NMR (nuclear magnetic resonance)); 2D IR (infrared) spectroscopy; 2D UV-Vis (Ultraviolet–Visible) spectroscopy; and 2D fluorescence spectroscopy helped identify how these combinations interact with soil minerals and SOM, providing insights into mechanisms like hydrogen bonding and chelation, which improve soil structure and microbial habitats [111]. Integrating HSs with organic amendments supports a circular economy by utilizing agricultural residues and reducing dependency on mineral fertilizers. This sustainable approach aligns with modern agricultural goals, including improving productivity while reducing environmental impact [15–112]. The recent literature highlights the complementary role of biochar and HSs in improving soil health and agricultural productivity. Biochar and HSs, both rich in organic matter, can work synergistically to enhance soil properties. HSs, which are formed through the humification process of organic materials, contribute to soil aggregation, increased nutrient retention, and improved water-holding capacity. When combined, biochar and HSs can enhance soil fertility and structure more effectively than when applied alone. Biochar provides a physical framework that stabilizes HSs in the soil, reducing their leaching potential and prolonging their beneficial effects on soil properties [113].

In addition, biochar's surface has functional groups that can bind to HSs, allowing for a more stable interaction between the two materials. This binding not only increases the effectiveness of both amendments but also reduces nutrient loss and enhances microbial activity in the soil. Furthermore, biochar can increase the availability of HSs to plants by improving the soil's ability to retain these substances. Studies have shown that the combined application of biochar and HSs can lead to enhanced soil carbon sequestration, improved soil microbial health, and increased crop yields, making it a promising strategy for sustainable agriculture [114]. Wiggins et al. [115] aimed to study the effects of cantaloupe (*Cucumis melo* L.) production in organic management systems influenced by HSs and biochar. Results evidenced that HSs alone and with biochar increased the number of fruits per plant in both research trials. Fruit diameter and fruit weight in cantaloupe treated with HSs were higher compared to biochar in the 2021 research trial. Authors concluded that HSs were able to provide benefits for organic cantaloupe production inducing significant enhancements in fruit number and quality. Additionally, the combined use of HA + FA was the best treatment strategy. Ji et al. [116] tested humic-like acids, derived from municipal sludge, on rice growth, soil fertility, and dissolved organic matter, using multi-chamber root box experiments. The application of artificial HAs resulted in a notable reduction in soil electrical conductivity, indicating improved soil properties. Additionally, there was a significant increase in dissolved organic carbon content within the root zone, alongside an enhancement in the fluorescence intensity of dissolved organic matter in the rhizosphere. While the proportion of specific components of dissolved organic matter exhibited only minor changes between the rhizosphere and near-rhizosphere zones, artificial HAs were found to promote the humification process of dissolved organic matter in both the near-rhizosphere and far-rhizosphere zones. These findings highlighted the distinct environmental effects of artificial HAs compared to conventional chemical fertilizers, demonstrating their potential as a sustainable alternative. With their capacity to enhance soil organic matter dynamics and improve soil health, artificial HAs hold significant promise for advancing agricultural productivity and environmental stewardship.

The integration of HSs into agricultural practices not only boosts crop yields but also significantly enhances crop quality. By promoting the efficient adsorption of essential nutrients, these substances enrich the nutrient profile of crops and support healthier, more vigorous plant growth. Additionally, HSs play a crucial role in improving plant resilience, enabling crops to better withstand environmental stresses, which further contributes to increased productivity [117]. Beyond their direct benefits to crop production, HSs align

seamlessly with the principles of sustainable agriculture. They enhance nutrient use efficiency, reducing reliance on mineral fertilizers, and thereby help lower greenhouse gas emissions and minimize nutrient run-off into aquatic ecosystems. These attributes highlight their dual role in fostering both agricultural productivity and environmental sustainability.

6. Environmental Ripple Effects of HSs

The chemical composition of HSs enables their participation in soil productivity [118]. The diverse and multifunctional nature of HSs underscores their importance in sustainable agriculture, environmental remediation, and climate change mitigation strategies.

The role of HSs in environmental remediation is multifaceted, contributing to the remediation of contaminated environments through several key mechanisms, including their ability to adsorb, stabilize, and mobilize contaminants, as well as enhance microbial activity and facilitate phytoremediation processes. Humic substances play a critical role in the adsorption of various contaminants, including heavy metals, organic pollutants, and pesticides. Through complexation and binding, HSs can stabilize these pollutants, preventing their mobility and toxicity in soil and water. For instance, HSs can bind to heavy metals, reducing their bioavailability and preventing them from leaching into groundwater [119,120]. HSs are also known to enhance the biodegradation of organic contaminants. They can improve the microbial environment by acting as carbon and energy sources for microorganisms, thus stimulating the microbial degradation of pollutants. This makes HSs an important tool in bioremediation processes, where they support the microbial breakdown of organic contaminants such as hydrocarbons [121]. Humic substances can enhance the effectiveness of phytoremediation, a process that uses plants to remove or degrade pollutants. HSs promote plant growth by improving soil structure, increasing nutrient availability, and enhancing root development. In turn, plants become more efficient in absorbing and removing contaminants from the environment [122]. In soil remediation, HSs assist in controlling pollutant bioavailability and toxicity. They can improve soil health by restoring microbial diversity and enhancing nutrient cycling, which is essential for soil recovery. HSs are particularly effective in the remediation of soils contaminated with heavy metals, hydrocarbons, and pesticides [123]. Recently, microplastic (MP) pollution, an anthropogenically driven threat to the biosphere, has emerged as a pressing global concern. A significant contributor to this issue is the accumulation of plastic residues in agricultural fields following crop cultivation. These residual plastics degrade over time into microplastics—minute particles less than 5 mm in diameter [124]. MPs profoundly affect soil's physicochemical properties, influencing porosity and enzymatic and microbial activities, and ultimately impacting plant growth and crop yield. Due to their pervasive nature, high surface area, and strong hydrophobicity, MPs act as vectors for the transport of harmful chemicals, including plasticizers, polycyclic aromatic hydrocarbons (PAHs), antibiotics, and potentially toxic elements (PTEs). Their mobility allows MPs to infiltrate deeper soil layers, posing a risk to groundwater quality. The detrimental effects of MPs on soil ecosystems include their impact on enzymatic activity, microbial diversity, plant health, and broader agricultural productivity through the spreading of toxic substances and their implications for soil and water systems. The study of microplastics (MPs) and Has remains underexplored, particularly in terms of their interactions. The findings of Klemes et al. [125] revealed that the average MP concentration in the soil surface layer (0–10 cm) was 190 $\pm$ 20 items/kg, which was significantly lower than concentrations in the 11–20 cm and 21–30 cm layers (211 $\pm$ 10 items/kg and 238 $\pm$ 18 items/kg, respectively). Additionally, larger MP particles were predominantly located in the surface layer (31.53–37.87%), whereas smaller particles were more abundant in deeper layers (73.23–100% in the 21–30 cm layer). HAs associated with MPs demonstrated a shift from low to high molecular weight

with increasing depth, from 0–10 cm to 21–30 cm. This molecular change may influence the vertical distribution of MPs. These findings enhance our understanding of MP migration and underscore the need to clarify MP-HA interactions to manage MP contamination in the environment. Despite variations in HSs, optimizing their use enhances plant growth, disease control, and crop productivity.

The effectiveness of HSs in heavy metal removal has been a focal point of environmental remediation studies, with aerobic HSs (AE-HSs) emerging as particularly effective. Through advanced analytical techniques such as spectral analysis and mass spectrometry, AE-HSs have been shown to contain functional groups like phenolic compounds, carboxylic acids, and aromatic structures. These groups play a critical role in forming humates or stable complexes, which facilitate the sequestration of heavy metals from contaminated soils [126]. Optimized AE-HSs demonstrated impressive removal efficiencies of 96.18% for cadmium, 82.75% for copper, 60.43% for zinc, and 41.66% for lead, highlighting their potential in targeted heavy metal remediation [127]. These findings underscore the potential of AE-HSs derived from agricultural residues, such as straw, in developing sustainable and effective strategies for soil remediation. These studies provide new insights into the preparation and application of straw-derived HSs, emphasizing their environmental significance. Beyond heavy metal removal, HSs influence the broader ecosystem through their role in carbon sequestration, nutrient cycling, and mitigating soil degradation. Their ability to interact with pollutants and enhance microbial activity makes them valuable not only for soil remediation but also for improving soil health and sustainability in agricultural practices [128]. This underscores the importance of further research into HSs to harness their full potential for addressing environmental challenges.

6.1. HSs and Soil Carbon Stabilization

The HSs are integral to soil organic carbon (SOC) storage and long-term carbon sequestration. Their formation through the humification process results in stable organic compounds that can persist in the soil for decades or even centuries, helping to mitigate atmospheric CO_2 levels [129,130]. This sequestration process reduces greenhouse gas emissions and supports global efforts to combat climate change. According to the "humus paradigm", HSs were assumed to be complex macromolecules forming the most stable soil organic matter fraction. The stable organic matter in soils can arise from both recalcitrant (slowly degradable) and labile (easily decomposable) organic compounds and occurs as these carbon inputs interact with soil minerals and aggregates, forming stable organic complexes over time. A key question is whether both labile and recalcitrant organic matter are capable of stabilizing in soil environments, despite differences in their chemical properties and decomposition rates. This phenomenon highlights complex interactions between organic carbon inputs and soil properties, suggesting a dual mechanism for carbon stabilization driven by both biochemical recalcitrance and physical–mineral interactions [131,132].

The stability of HSs arises from their complex aromatic and aliphatic structures, which resist microbial decomposition. Practices such as adding compost, biochar, and organic amendments enhance humic substance content, improving the soil's carbon storage potential [133]. Research shows that soils rich in HSs can store up to 30% more carbon compared to degraded soils [7]. Innovative soil management strategies incorporating hydrophobic HSs are emerging as effective solutions to enhance the biological stability of soil organic matter and mitigate CO_2 emissions from agricultural soils. These management approaches capitalize on the unique properties of hydrophobic HSs, which interact with soil particles to create a more stable and resilient organic matter matrix, thereby protecting

it from microbial decomposition [112]. This stabilization reduces the rate of organic matter mineralization, thereby curbing CO_2 emissions into the atmosphere.

The HSs increase microbial stability and mitigate rapid soil carbon breakdown, contributing significantly to climate change mitigation. Humic substances serving as energy sources for soil microorganisms promote their growth and metabolism. This increased microbial activity leads to more efficient organic matter decomposition, resulting in the stabilization and sequestration of carbon in the soil as stable humic compounds [134]. The microorganisms break down organic material into humus, which is more resistant to further decomposition, effectively locking carbon in the soil for longer periods.

Recent research supports their role in improving soil health by stabilizing organic matter and enhancing the overall carbon storage capacity. Furthermore, hydrophobic HSs improve soil structure by promoting the aggregation of soil particles. This aggregation enhances soil porosity and aeration, allowing for better water infiltration while reducing soil erosion risks and minimizing the release of deep carbon pools into the atmosphere [15]. The HSs reduce soil respiration rates, thereby contributing to the stabilization of organic carbon and further limiting greenhouse gas emissions [96]. The role of HSs in carbon stabilization can help to mitigate greenhouse gas emissions, aligning sustainable agriculture with global climate goals [12]. Recent experimental evidence from Spietz et al. [135] provides additional insights into the carbon sequestration potential of hydrophobic acid substances and their salts (ammonium and potassium). Their study demonstrated that HAs exhibit a CO_2 absorption capacity of up to 10.9 g CO_2 per kg, with the ammonium variant exhibiting higher CO_2 absorption rates compared to potassium salts. This suggests that HS compounds have a dual role: they not only form complex structures capable of sequestering carbon and preventing its emission, but also act as effective salts when applied to soil, enhancing the soil's ability to bind and store atmospheric CO_2. These findings highlighted the versatility of HSs, emphasizing their role as both natural carbon sinks and active soil amendments that can be used to combat climate change through innovative management practices. The combination of biological, structural, and chemical mechanisms underscores their potential for long-term climate change mitigation efforts.

6.2. HSs as Strategy to Combat Environmental Pollution

In agricultural systems, the application of HSs has been shown to reduce the leaching of nutrients such as nitrates (NO_3^-) and phosphates (PO_4^{3-}) into groundwater. HSs contain negatively charged functional groups that interact with cations, enhancing the soil's ability to retain and exchange nutrients (Table 5). This increased CEC indirectly helps to retain anions like nitrates and phosphates, reducing their movement and leaching. Additionally, HSs can form electrostatic bonds with nitrates and phosphates, especially in alkaline soils, which reduces the mobility of these nutrients and prevents leaching. This binding helps keep nutrients in the root zone for better plant uptake. By promoting soil aggregation, HSs improve soil structure, creating stable microenvironments where nutrients are less prone to leaching. This enhanced aggregation improves nutrient retention, especially during rainfall or irrigation. HSs stimulate microbial activity, which aids in nutrient cycling and the transformation of organic matter into stable forms. This process reduces nitrate leaching by converting excess nitrates into less mobile forms and enhances phosphate availability for plants. The formation of stable organic complexes between HSs and both cations and anions reduce the mobility of nutrients like nitrates and phosphates. These complexes help retain nutrients in the soil, reducing nutrient loss and improving long-term nutrient availability [136]. By enhancing nutrient retention and uptake efficiency, HSs help minimize the need for mineral fertilizers, thereby reducing chemical inputs and their associated environmental impact (Table 5). This is particularly important for addressing

issues like eutrophication in freshwater bodies caused by nutrient runoff. Furthermore, organic wastes combined with HSs can further enhance this effect by creating stable organic matter complexes that trap residual agricultural pollutants, contributing to a circular economy approach in agriculture [110].

Additionally, HSs influence microbial activity by promoting beneficial microbial communities while suppressing harmful ones, further stabilizing the soil ecosystem and limiting the degradation pathways that lead to environmental pollution [137]. HSs can degrade certain organic pollutants through interactions with soil microorganisms. They facilitate the breakdown of pesticides and hydrocarbons, contributing to soil remediation and the restoration of contaminated lands [94]. This makes HSs valuable tools in bioremediation practices aimed at reducing pollution in agricultural and industrial soils (Table 5). HSs can mitigate the bioavailability and mobility of harmful contaminants, such as heavy metals and pesticides, by binding with these pollutants through complexation and chelation processes. HSs contain functional groups, such as carboxyl, hydroxyl, and phenolic groups, that can form strong complexes with heavy metals like lead (Pb), cadmium (Cd), arsenic (As), and mercury (Hg). These functional groups bind with the metals, making them less available for uptake by plants and microorganisms. HSs enhancing soil aggregation, by promoting the formation of stable aggregates, trap heavy metals within aggregate structure, reducing their mobility in the soil profile [138].

This interaction prevents these compounds from leaching into water systems and reduces their toxicity in the environment. Recent studies emphasize HSs' ability to sequester pollutants in soil matrices, enhancing the capacity of soils to retain and immobilize these compounds, thereby reducing their harmful impacts.

Innovative treatments, such as combining HSs with biochar or other organic amendments, are being explored to enhance their ability to remove contaminants from soils and water through adsorption and retention mechanisms. This highlights their versatility and potential to serve as multifunctional agents for sustainable agriculture and climate resilience [139].

The HSs have recently been recognized as having significant effects on the environmental behavior of antibiotic resistance genes (ARGs), and this area is emerging as a critical research focus [140]. The interaction of HSs with ARGs is complex, as these interactions are influenced by various environmental factors, including the presence of coexisting compounds such as antibiotics, heavy metals, and other organic particles. While the mechanisms remain partially understood, HSs have demonstrated the potential to inhibit the expression of ARGs and facilitate their removal from environmental systems, both directly and indirectly.

Research suggests that HSs interact with ARGs through various mechanisms, including electrostatic interactions, hydrophobic effects, and hydrogen bonding, all of which can alter the mobility, bioavailability, and genetic expression of ARGs in the soil–water interface. Additionally, microbial community shifts induced by the presence of HSs can impact the evolution and fate of ARGs within ecosystems. Despite these promising findings, the interaction between HSs, ARGs, and environmental co-contaminants remains underexplored, and the mechanisms of their interactions are not yet fully elucidated. Moreover, studies are sparse regarding the specific correlation between microbial populations, the environmental presence of ARGs, and HSs effects under various environmental conditions [141].

Overall, the application of HSs demonstrates promise in addressing key environmental challenges by improving soil health, reducing pollutant mobility, and fostering nutrient cycling, all while supporting sustainable agricultural practices [111].

Table 5. Role of HSs in environmental remediation technologies and their effectiveness.

Contaminant	Remediation Technology	Role of HSs	Effectiveness/Results	References
Hydrocarbons (pesticides, PAHs)	Biodegradation and Bioremediation	HSs improve the microbial environment, stimulating the biodegradation of organic contaminants through microorganisms.	Increased microbial activity that stimulates the degradation of organic contaminants like hydrocarbons and pesticides, facilitating bioremediation.	[123]
Microplastics (MP)	Adsorption and Mobilization	HSs can bind to microplastics, influencing their vertical distribution in soil and interacting with harmful chemicals.	MP-HS interactions showed a shift from low to high molecular weight with increasing depth.	[125]
Heavy Metals (Cd, Cu, Pb)	Adsorption and Stabilization	HSs bind and stabilize heavy metals, reducing their bioavailability and preventing their mobility in soil and water.	96.18% removal for cadmium, 82.75% for copper, 60.43% for zinc, 41.66% for lead (aerobic HSs).	[126,127]
Nutrients (NO_3^-, PO_4^{3-})	Leaching Prevention	HSs improve nutrient retention in soil and reduce the leaching of nitrates and phosphates.	Reduced nutrient leaching and improved plant uptake efficiency, reducing the need for synthetic fertilizers.	[136]
Heavy Metals, Pesticides	Combination with Biochar or Other Organic Amendments	HSs, combined with biochar or other organic amendments, improve adsorption and removal of contaminants from soil and water.	Greater ability to remove heavy metals and pesticides, reducing their mobility and toxicity in the environment.	[139]
Antibiotic Resistance Genes (ARGs)	Interaction with Microorganisms	HSs interact with antibiotic resistance genes (ARGs), influencing their mobility, bioavailability, and genetic expression.	Potential inhibition of ARG expression and facilitation of their removal, though studies are still limited.	[140,141]

6.3. The Economics Benefits of Using HSs in Agriculture

HSs have gained significant attention due to their potential to improve soil health, increase crop yields, and enhance nutrient efficiency. However, the profitability of using HSs in agriculture depends on several factors, including application rates, crop types, soil conditions, and regional agricultural practices. HSs improve soil structure, increase cation exchange capacity, and enhance nutrient and water retention, which results in healthier soils. This can reduce the need for mineral fertilizers, leading to cost savings in the long run. HSs also help in the immobilization of heavy metals and toxins, thus preventing soil degradation and reducing the risk of contamination, which can be costly to remediate. HSs can increase plant growth and productivity by improving nutrient availability and promoting better root development. Studies have shown that the application of HSs can lead to an increase in crop yields by 10–20%, depending on the crop type, soil conditions, and application methods [107,141]. These yield improvements translate to higher profits for farmers. One of the key advantages of using HSs is their ability to enhance nutrient retention in the soil, reducing the need for chemical fertilizers. This can be particularly beneficial in areas where fertilizer costs are high or where the environmental impact of excessive fertilizer use is a concern. By improving nutrient uptake efficiency, HSs reduce the need for frequent or high-input fertilizer applications. The application of HSs can improve carbon sequestration in soil, thus contributing to climate change mitigation efforts. Soils treated with HSs have higher organic matter content, which is important for long-term sustainability and resilience against climate change. Farmers can benefit from potential carbon credit schemes or subsidies aimed at promoting environmentally friendly farming practices [142]. The costs associated with HSs vary depending on the source (e.g., extracted from leonardite, composted organic matter, or biochar). Commercial HS products can

range from USD 50 to USD 300 per ton, depending on the concentration and formulation. While this represents an upfront cost for farmers, the long-term benefits of improved soil health and productivity can offset these expenses. The recommended application rate of HSs depends on soil type, crop, and specific agricultural practices [143]. In organic or regenerative farming systems, where reducing chemical inputs is a priority, HSs offer a cost-effective alternative that aligns with sustainability goals [144]. The quality and consistency of HS products can vary, and farmers must ensure that they are purchasing high-quality, well-characterized materials that contain effective concentrations of active humic compounds. Poor-quality products may not deliver the desired benefits and could lead to financial losses [145]. One of the challenges faced by farmers in adopting HSs is the lack of awareness and knowledge about their benefits and proper application methods. Many farmers may be hesitant to adopt new practices without clear evidence of profitability [146]. Education and extension services are essential to ensure that farmers understand the value and potential of HSs. The effectiveness of HSs can vary depending on soil type, climate, and local agricultural practices. In some regions with well-developed soil management systems, the benefits of HSs may be more apparent, while in others, the costs may outweigh the benefits if the local soil conditions do not align with HS properties. The adoption of HSs is growing globally, particularly in regions with large-scale, intensive agricultural practices, such as parts of Europe, North America, and Asia. However, the economic viability is more pronounced in regions where soil degradation is a significant issue, such as in parts of Africa and Latin America, where improving soil organic matter content is critical for sustainable farming [147]. In short, while the initial costs of applying HSs may be high, their long-term benefits, such as increased crop yields, reduced fertilizer usage, and improved soil health, make them a potentially profitable investment for farmers. The economics of HSs depend on proper application rates, soil conditions, and the choice of crops. With the growing focus on sustainable agriculture and environmental protection, HSs offer an economically viable solution to improve soil fertility, enhance productivity, and reduce reliance on chemical inputs.

7. Conclusions, Challenges, and Future Directions

In conclusion, HSs, comprising HAs, FAs, and HU, hold significant promise as plant biostimulants to address the challenges of modern agriculture. These organic compounds play a pivotal role in enhancing crop productivity and sustainability by improving nutrient uptake and utilization, bolstering stress tolerance in plants, and influencing hormonal regulation. The advantages of using HSs are substantiated by various case studies across different crops, showcasing increased yields, improved crop quality, and reduced environmental impact. By facilitating nutrient absorption, promoting soil health, and enhancing stress resilience, HSs align with the goals of sustainable agriculture. However, challenges and limitations, including variations in commercial product quality, application methods, and costs, must be addressed to maximize the benefits of HSs for sustainable agricultural purposes. Rather than focusing on the traditional determination of the precise structure of HSs—a goal for which no universally accepted model has yet been achieved—the emphasis shifts toward mapping relationships and studying patterns. This approach involves moving away from purely quantitative methods toward a qualitative analysis, prioritizing the exploration of processes, environmental interactions, and plant interactions over rigid structural definitions. From this perspective, examining the balance between hydrophilic and hydrophobic components becomes crucial for understanding the environmental reactivity of HSs. This reactivity arises from the organizational processes involved in the aggregation of humic matter into macroscopic structures. Such aggregates exhibit collective behavior characterized by ordered patterns in large-scale systems while remaining less defined at

the microscopic level. This macroscopic approach underscores the dynamic and emergent properties of HSs, offering valuable insights into their functional roles in environmental and biological contexts. Developing standardized guidelines for their application can help maximize their benefits, ensuring a more resilient and environmentally responsible food production system for the 21st-century growing global population.

Despite significant progress in understanding the role of HSs in biological and ecological processes, key knowledge gaps persist regarding their mechanistic functions. The complexity and heterogeneity of HS composition present challenges in identifying specific molecular interactions and pathways responsible for their effects. Limited clarity exists on how HSs influence cellular signaling, hormone-like activities, and nutrient transport at a molecular level. Additionally, variations in the bioactivity of HSs across different environmental contexts remain poorly understood. Addressing these gaps is essential for fully harnessing the potential of HSs in applications such as agriculture, environmental restoration, and biotechnology.

Author Contributions: Conceptualization, A.M. (Adele Muscolo); writing—original draft preparation, A.M. (Adele Muscolo), and A.M. (Angela Maffia); formal analysis, A.M. (Adele Muscolo) and A.M. (Angela Maffia), investigation, A.M. (Adele Muscolo) and A.M. (Angela Maffia), review and editing, S.N. (Serenella Nardi) and A.M. (Adele Muscolo); supervision, A.M. (Adele Muscolo); software, F.M. (Federica Marra); C.M. (Carmelo Mallamaci) and M.O. (Mariateresa Oliva); project administration, A.M. (Adele Muscolo); funding acquisition, A.M. (Adele Muscolo). All authors have read and agreed to the published version of the manuscript.

Funding: This study was financially supported by European Commission, project LIFE20ENV/IT/000,229–LIFE RecOrgFert PLUS, LIFE Programme.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. FAO. *The State of the World's Land and Water Resources for Food and Agriculture 2021: Systems at Breaking Point*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2021.
2. Rockström, J.; Edenhofer, O.; Gaertner, J.; DeClerck, F. Planet-Proofing the Global Food System. *Nat. Food* **2020**, *1*, 3–5. [CrossRef]
3. Pretty, J.; Benton, T.G.; Bharucha, Z.P.; Dicks, L.V.; Flora, C.B.; Godfray, H.C.J.; Goulson, D.; Hartley, S.; Lampkin, N.; Morris, C.; et al. Global Assessment of Agricultural System Redesign for Sustainable Intensification. *Nat. Sustain.* **2020**, *3*, 830–840. [CrossRef]
4. Smith, P.; Soussana, J.; Angers, D.; Schipper, L.; Chenu, C.; Rasse, D.P.; Batjes, N.H.; Van Egmond, F.; McNeill, S.; Kuhnert, M.; et al. How to measure, report and verify soil carbon change to realize the potential of soil carbon sequestration for atmospheric greenhouse gas removal. *Glob. Chang. Biol.* **2019**, *26*, 219–241. [CrossRef] [PubMed]
5. Kassam, A.; Friedrich, T.; Derpsch, R. Global Achievements in Soil Conservation and the Role of Conservation Agriculture. *Int. Soil Water Conserv. Res.* **2021**, *9*, 217–230.
6. Jin, X.; Zhang, Y.; Zhou, J. Advances in Precision Agriculture for Sustainable Farming: A Review. *Agronomy* **2022**, *12*, 412.
7. Canellas, L.P.; Canellas, N.O.A.; Olivares, F.L.; Piccolo, A. Humic Substances in the Era of Soil Health: A Critical Review. *J. Soils Sediments* **2021**, *21*, 1562–1576.
8. Rosa, J.; Silva, R.F.; Canellas, L.P. Humic Acids and Sustainable Nutrient Management in Agriculture. *J. Plant Nutr.* **2022**, *45*, 1175–1190.
9. Abid, M.; Batool, T.; Siddique, G.; Ali, S.; Binyamin, R.; Shahid, M.J.; Rizwan, M.; Alsahli, A.A.; Alyemeni, M.N. Integrated nutrient management enhances soil quality and crop productivity in Maize-Based cropping system. *Sustainability* **2020**, *12*, 10214. [CrossRef]
10. Guo, X.-X.; Liu, H.-T.; Wu, S.-B. Humic Substances developed during organic waste composting: Formation mechanisms, structural properties, and agronomic functions. *Sci. Total Environ.* **2019**, *662*, 501–510. [CrossRef] [PubMed]
11. Kłeczek, A. Agricultural Use of Natural Biostimulants—Humic Substances: A Review. *Deleted J.* **2022**, *24*, 1–14. [CrossRef]

12. Nardi, S.; Ertani, A.; Francioso, O. Soil–root cross-talking: The role of Humic Substances. *J. Plant Nutr. Soil Sci.* **2016**, *180*, 5–13. [CrossRef]
13. Tamburini, G.; Bommarco, R.; Wanger, T.C.; Kremen, C.; Van Der Heijden, M.G.A.; Liebman, M.; Hallin, S. Agricultural diversification promotes multiple ecosystem services without compromising yield. *Sci. Adv.* **2020**, *6*, eaba1715. [CrossRef]
14. Dainese, M.; Martin, E.A.; Aizen, M.A.; Albrecht, M.; Bartomeus, I.; Bommarco, R.; Carvalheiro, L.G.; Chaplin-Kramer, R.; Gagic, V.; Garibaldi, L.A.; et al. A global synthesis reveals biodiversity-mediated benefits for crop production. *Sci. Adv.* **2019**, *5*, eaax0121. [CrossRef] [PubMed]
15. García, A.C.; Olaetxea, M.; Santos, L.A.; Mora, V.; Baigorri, R.; Fuentes, M.; Zamarreño, A.M.; Berbara, R.L.L.; Garcia-Mina, J.M. Involvement of Hormone- and ROS-Signaling Pathways in the Beneficial Action of Humic Substances on Plants Growing under Normal and Stressing Conditions. *BioMed Res. Int.* **2016**, *2016*, 3747501. [CrossRef] [PubMed]
16. Foley, J.A.; Ramankutty, N.; Brauman, K.A.; Cassidy, E.S.; Gerber, J.S.; Johnston, M.; Mueller, N.D.; O'Connell, C.; Ray, D.K.; West, P.C.; et al. Solutions for a cultivated planet. *Nature* **2011**, *478*, 337–342. [CrossRef]
17. Canellas, L.P.; da Silva, R.M.; Busato, J.G.; Olivares, F.L. Humic Substances and plant abiotic stress adaptation. *Chem. Biol. Technol. Agric.* **2024**, *11*, 66. [CrossRef]
18. Yang, F.; Tang, C.; Antonietti, M. Natural and artificial Humic Substances to manage minerals, ions, water, and soil microorganisms. *Chem. Soc. Rev.* **2021**, *50*, 6221–6239. [CrossRef]
19. BerzeliusJöns Jakob. *Försök att Bestämma Det Ämne Som Gör Det Svenska Vattnet Mörkfärgat*; Royal Swedish Academy of Sciences: Stockholm, Sweden, 1807.
20. Waksman, S.A. *Humus: Origin, Chemical Composition, and Importance in Nature*; Williams & Wilkins: Baltimore, MD, USA, 1932.
21. Kononova, M.V. *Soil Organic Matter: Its Nature, Its Role in Soil Formation and in Soil Fertility*; State Publishing House of Agricultural Literature: Moscow, Russia, 1947.
22. Stevenson, F.J. *Humus Chemistry: Genesis, Composition, Reactions*; John Wiley & Sons: New York, NY, USA, 1972.
23. Swift, R.S. Molecular Weight, Size, Shape, and Charge Characteristics of Humic Substances: Some Basic Considerations. In *Humic Substances II*; MacCarthy, H.H.B., Ed.; Wiley: New York, NY, USA, 1989.
24. International Humic Substances Society (IHS). Definition of Humic Substances. 2000. Available online: https://humic-substances.org (accessed on 11 September 1981).
25. Chen, Y.; Zhang, L.; Wang, S.; Zeng, Q.; Xia, Q.; Li, R.; Guo, D.; Pan, Z.; Dong, H. Combined effects of Humic Substances and clay minerals on U(VI) bioreduction. *Geochim. Cosmochim. Acta* **2022**, *338*, 181–198. [CrossRef]
26. MacCarthy, P. The principles of Humic Substamces: An introduction to the first principle. In *Humic Substances: Structures, Models and Functions*; Royal Society of Chemistry: London, UK, 2001; pp. 19–30. [CrossRef]
27. Perminova, I.V.; Frimmel, F.H.; Kudryavtsev, A.V.; Kulikova, N.A.; Abbt-Braun, G.; Hesse, S.; Petrosyan, V.S. Molecular Weight Characteristics of Humic Substances from Different Environments As Determined by Size Exclusion Chromatography and Their Statistical Evaluation. *Environ. Sci. Technol.* **2003**, *37*, 2477–2485. [CrossRef]
28. Piccolo, A. The supramolecular structure of Humic Substances: A novel understanding of humus chemistry and implications in soil science. In *Advances in Agronomy*; Elsevier: Amsterdam, The Netherlands, 2002; pp. 57–134. [CrossRef]
29. Piccolo, A.; Conte, P. Molecular size of Humic Substances. Supramolecular associations versus macromolecular polymers. *Adv. Environ. Res.* **2000**, *3*, 508–521.
30. Volikov, A.B.; Kholodov, V.A.; Kulikova, N.A.; Philippova, O.I.; Ponomarenko, S.A.; Lasareva, E.V.; Parfyonova, A.M.; Hatfield, K.; Perminova, I.V. Silanized Humic Substances act as hydrophobic modifiers of soil separates inducing formation of water-stable aggregates in soils. *Catena* **2016**, *137*, 229–236. [CrossRef]
31. Sarlaki, E.; Kianmehr, M.H.; Marzban, N.; Shafizadeh, A.; Tajuddin, S.A.F.S.A.; Hu, S.; Tabatabaei, M.; Aghbashlo, M. Advances and challenges in humic acid production technologies from natural carbonaceous material wastes. *Chem. Eng. J.* **2024**, *498*, 155521. [CrossRef]
32. de Melo, R.E.; de Abreu, E.D.; Barbosa, E.D.; da Silva, A.E.B.; da Silva, C.B.; de Souza Júnior, J.H.; Silva Neto, C.P.; da Silva, J.R. Humic Substances extracted from organic compounds and their influence on the morphophysiological characteristics of tomato seedlings. *Rev. Delos* **2023**, *16*, 734–751. [CrossRef]
33. Krumins, J.; Klavins, M. Characterisation of humic acids in boreal mires depending on a peat type. *Boreal Environ. Res.* **2022**, *27*, 1.
34. Kononova, M.M. *Soil Organic Matter*; Academy of Sciences of the USSR: Moscow, Russia, 1963.
35. Zavarzina, A.G.; Danchenko, N.N.; Demin, V.V.; Artemyeva, Z.S.; Kogut, B.M. Humic Substances: Hypotheses and Reality (A Review). *Eurasian Soil Sci.* **2021**, *54*, 1826–1854. [CrossRef]
36. Senesi, N.; Xing, B.; Huang, P.M. *Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems*; John Wiley & Sons: Hoboken, NJ, USA, 2009. [CrossRef]
37. Fragouli, P.G.; Roulia, M.; Vassiliadis, A.A. Macromolecular Size and Architecture of Humic Substances Used in the Dyes' Adsorptive Removal from Water and Soil. *Agronomy* **2023**, *13*, 2926. [CrossRef]
38. Gerke, J. Concepts and misconceptions of HS as the stable part of soil organic matter: A review. *Agronomy* **2018**, *8*, 76. [CrossRef]

39. Michalska, J.; Turek-Szytow, J.; Dudło, A.; Surmacz-Górska, J. Characterization of Humic Substances recovered from the sewage sludge and validity of their removal from this waste. *EFB Bioecon. J.* **2022**, *2*, 100026. [CrossRef]
40. Schnitzer, M. Characterization of humic constituents by spectroscopy. In *Soil Biochemistry*; McLaren, A.D., Ed.; Marcel Dekker: New York, NY, USA, 1971; Volume 2, p. 60.
41. Albers, C.N.; Banta, G.T.; Jacobsen, O.S.; Hansen, P.E. Characterization and structural modelling of Humic Substances in field soil displaying significant differences from previously proposed structures. *Eur. J. Soil Sci.* **2008**, *59*, 693–705. [CrossRef]
42. Burdon, J. Are the Traditional Concepts of the Structures of Humic Substances Realistic? *Soil Sci.* **2001**, *166*, 752–769. [CrossRef]
43. Tiwari, J.; Ramanathan, A.; Bauddh, K.; Korstad, J. Humic Substances: Structure, function and benefits for agroecosystems—A review. *Pedosphere* **2022**, *33*, 237–249. [CrossRef]
44. Zou, J.; Zhang, H.; Yue, D.; Huang, J. Is the traditional alkali extraction method valid in isolating chemically distinct humic acid? *Chem. Eng. J. Adv.* **2021**, *6*, 100077. [CrossRef]
45. Machado, W.; Franchini, J.C.; De Fátima Guimarães, M.; Filho, J.T. Spectroscopic characterization of humic and fulvic acids in soil aggregates, Brazil. *Heliyon* **2020**, *6*, e04078. [CrossRef] [PubMed]
46. Jastrow, J.D.; Miller, R.M. Soil Aggregate Stabilization and Carbon Sequestration: Feedbacks through Organomineral Associations. In *Soil Processes and the Carbon Cycle*; CRC Press: Boca Raton, FL, USA, 2018; pp. 207–223. [CrossRef]
47. Sharma, B.; Dinesh, R. Humic Substances in soil and their role in soil fertility and plant growth. *Int. J. Agric. Environ. Biotechnol.* **2017**, *10*, 537–545.
48. Lumactud, R.A.; Gorim, L.Y.; Thilakarathna M., S. Impacts of humic-based products on the microbial community structure and functions toward sustainable agriculture. *Front. Sustain. Food Syst.* **2022**, *6*. Available online: https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2022.977121 (accessed on 28 January 2025). [CrossRef]
49. Rani, J.; Kumari, S.; Paul, B. Extraction and chemical characterization of humic acid produced from lignite coals of arid region of Gujarat, Western India. *Sci. Rep.* **2024**, *14*. [CrossRef] [PubMed]
50. Peña-Méndez, E.M.; Havel, J.; Patočka, J. Humic Substances—Compounds of still unknown structure: Applications in agriculture, industry, environment, and biomedicine. *J. Appl. Biomed.* **2005**, *3*, 13–24. [CrossRef]
51. Canellas, L.P.; Canellas, N.O.A.; da Silva, R.M.; Spaccini, R.; Mota, G.P.; Olivares, F.L. Biostimulants Using Humic Substances and Plant-Growth-Promoting Bacteria: Effects on Cassava (Manihot esculentus) and Okra (Abelmoschus esculentus) Yield. *Agronomy* **2023**, *13*, 80. [CrossRef]
52. Canellas, L.P.; Olivares, F.L. Physiological responses to Humic Substances as plant growth promoter. *Chem. Biol. Technol. Agric.* **2014**, *1*, 3. [CrossRef]
53. Du Jardin, P. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* **2015**, *196*, 3–14. [CrossRef]
54. Shahabivand, S.; Padash, A.; Aghaee, A.; Nasiri, Y.; Rezaei, P.F. Plant biostimulants (Funneliformis mosseae and Humic Substances) rather than chemical fertilizer improved biochemical responses in peppermint. *Plant Physiol.* **2018**, *8*, 2333–2344.
55. Vikram, N.; Sagar, A.; Gangwar, C.; Husain, R.; Kewat, R.N. Properties of humic acid substances and their effect in soil quality and plant health. In *Humus and Humic Substances-Recent Advances*; IntechOpen: London, UK, 2022. [CrossRef]
56. Rose, M.T.; Patti, A.F.; Little, K.R.; Brown, A.L.; Jackson, W.R.; Cavagnaro, T.R. A Meta-Analysis and review of Plant-Growth response to Humic Substances. *Adv. Agron.* **2014**, *124*, 37–89. [CrossRef]
57. Quaggiotti, S.; Ruperti, B.; Pizzeghello, D.; Francioso, O.; Tugnoli, V.; Nardi, S. Effect of low molecular size Humic Substances on nitrate uptake and expression of genes involved in nitrate transport in maize (*Zea mays* L.). *J. Exp. Bot.* **2004**, *55*, 803–813. [CrossRef]
58. Derkowska, E.; Paszt, L.S.; Dyki, B.; Sumorok, B. Assessment of mycorrhizal frequency in the roots of fruit plants using different dyes. *Adv. Microbiol.* **2015**, *5*, 54–64. [CrossRef]
59. Afifi, M.M.I.; Ismail, A.M.; Kamel, S.M.; Essa, T.A. Humic Substances: A Powerful Tool for Controlling Fusarium Wilt Disease and Improving the Growth of Cucumber Plants. *J. Plant Pathol.* **2017**, *99*, 61–67. [CrossRef]
60. Yigit, F.; Dikilitas, M. Effect of Humic Acid Applications on the Root-Rot Diseases Caused by *Fusarium* spp. on Tomato Plants. *Plant Pathol. J.* **2008**, *7*, 179–182. [CrossRef]
61. Dordas, C. Role of nutrients in controlling plant diseases in sustainable agriculture. A review. *Agron. Sustain. Dev.* **2008**, *28*, 33–46. [CrossRef]
62. Wei, S.; Li, Z.; Sun, Y.; Zhang, J.; Ge, Y.; Li, Z. A comprehensive review on biomass humification: Recent advances in pathways, challenges, new applications, and perspectives. *Renew. Sustain. Energy Rev.* **2022**, *170*, 112984. [CrossRef]
63. De Hita, D.; Fuentes, M.; Zamarreño, A.M.; Ruiz, Y.; Garcia-Mina, J.M. Culturable bacterial endophytes from sedimentary humic Acid-Treated plants. *Front. Plant Sci.* **2020**, *11*, 837. [CrossRef]
64. Matuszak-Slamani, R.; Bejger, R.; Włodarczyk, M.; Kulpa, D.; Sienkiewicz, M.; Gołębiowska, D.; Skórska, E.; Ukalska-Jaruga, A. Effect of Humic Acids on Soybean Seedling Growth under Polyethylene-Glycol-6000-Induced Drought Stress. *Agronomy* **2022**, *12*, 1109. [CrossRef]

65. Muscolo, A.; Sidari, M.; Nardi, S. Humic substance: Relationship between structure and activity. Deeper information suggests univocal findings. *J. Geochem. Explor.* **2013**, *129*, 103–111. [CrossRef]
66. Maffia, A.; Marra, F.; Canino, F.; Battaglia, S.; Mallamaci, C.; Oliva, M.; Muscolo, A. Humic Substaces from Waste-Based Fertilizers for Improved Soil Fertility. *Agronomy* **2024**, *14*, 2657. [CrossRef]
67. Pukalchik, M.; Kydralieva, K.; Yakimenko, O.; Fedoseeva, E.; Terekhova, V. Outlining the potential role of humic products in modifying biological properties of the Soil—A review. *Front. Environ. Sci.* **2019**, *7*, 80. [CrossRef]
68. García, A.; de Souza, L.; Pereira, M.; Castro, R.N.; García-Mina, J.M.; Zonta, E.; Lisboa, F.J.G.; Berbara, R.L.L. Structure-Property-Function Relationship in Humic Substances to Explain the Biological Activity in Plants. *Sci. Rep.* **2016**, *6*, 20798. [CrossRef] [PubMed]
69. Johnson, J.M.-F.; Franzluebbers, A.J.; Weyers, S.L.; Reicosky, D.C. Agricultural opportunities to mitigate greenhouse gas emissions. *Environ. Pollut.* **2007**, *150*, 107–124. [CrossRef] [PubMed]
70. Gorlenko, M.V.; Yakimenko, O.S.; Golichenkov, M.V.; Kostina, N.V. Functional biodiversity of soil microbe colonies affected by organic substrates of different kinds. *Mosc. Univ. Soil Sci. Bull.* **2012**, *67*, 71–78. [CrossRef]
71. Li, C.; Xiong, Y.; Zou, J.; Dong, L.; Ren, P.; Huang, G. Impact of biochar and lignite-based amendments on microbial communities and greenhouse gas emissions from agricultural soil. *Vadose Zone J.* **2021**, *20*, e20105. [CrossRef]
72. De Andrade Da Silva, M.S.R.; De Melo Silveira Dos Santos, B.; De Andrade Da Silva, C.S.R.; De Andrade Da Silva, C.S.R.; De Sousa Antunes, L.F.; Santos, R.M.D.; Santos, C.H.B.; Rigobelo, E.C. Humic Substances in combination with plant Growth-Promoting bacteria as an alternative for sustainable agriculture. *Front. Microbiol.* **2021**, *12*, 719653. [CrossRef]
73. Dong, L.; Córdova-Kreylos, A.L.; Yang, J.; Yuan, H.; Scow, K.M. Humic acids buffer the effects of urea on soil ammonia oxidizers and potential nitrification. *Soil Biol. Biochem.* **2009**, *41*, 1612–1621. [CrossRef]
74. Puglisi, E.; Fragoulis, G.; Ricciuti, P.; Cappa, F.; Spaccini, R.; Piccolo, A.; Trevisan, M.; Crecchio, C. Effects of a humic acid and its size-fractions on the bacterial community of soil rhizosphere under maize (*Zea mays* L.). *Chemosphere* **2009**, *77*, 829–837. [CrossRef]
75. Van Trump, J.I.; Wrighton, K.C.; Thrash, J.C.; Weber, K.A.; Andersen, G.L.; Coates, J.D. Humic Acid-Oxidizing, Nitrate-Reducing bacteria in agricultural soils. *mBio* **2011**, *2*, e00044-11. [CrossRef] [PubMed]
76. Puglisi, E.; Pascazio, S.; Suciu, N.; Cattani, I.; Fait, G.; Spaccini, R.; Crecchio, C.; Piccolo, A.; Trevisan, M. Rhizosphere microbial diversity as influenced by humic substance amendments and chemical composition of rhizodeposits. *J. Geochem. Explor.* **2012**, *129*, 82–94. [CrossRef]
77. Li, Y.; Fang, F.; Wei, J.; Wu, X.; Cui, R.; Li, G.; Zheng, F.; Tan, D. Humic acid fertilizer improved soil properties and soil microbial diversity of continuous cropping peanut: A three-year experiment. *Sci. Rep.* **2019**, *9*, 12014. [CrossRef]
78. Trevisan, S.; Francioso, O.; Quaggiotti, S.; Nardi, S. Humic substances biological activity at the plant-soil interface. *Plant Signal. Behav.* **2010**, *5*, 635–643. [CrossRef] [PubMed]
79. Chen, C.; Yao, X.; Li, Q.X.; Wang, Q.; Liang, J.; Zhang, S.; Ming, J.; Liu, Z.; Deng, J.; Yoza, B.A. Turf soil enhances treatment efficiency and performance of phenolic wastewater in an up-flow anaerobic sludge blanket reactor. *Chemosphere* **2018**, *204*, 227–234. [CrossRef]
80. Schoebitz, L.; Niwagaba, C.B.; Strande, L. SFD promotion initiative Kampala Uganda final report. Eawag/Sandec. 2016. Available online: https://www.susana.org/_resources/documents/default/3-2593-7-1478269444.pdf (accessed on 6 June 2016).
81. Muscolo, A.; Panuccio, M.R.; Sidari, M.; Nardi, S. The effects of Humic Substances on Pinus callus are reversed by 2,4-dichlorophenoxy acetic acid. *J. Chem. Ecol.* **2005**, *31*, 577–590. [CrossRef]
82. Russell, L.; Stokes, A.R.; Macdonald, H.; Muscolo, A.; Nardi, S. Stomatal Responses to Humic Substances and Auxin are Sensitive to Inhibitors of Phospholipase A2. *Plant Soil* **2006**, *283*, 175–185. [CrossRef]
83. Jindo, K.; Martim, S.A.; Navarro, E.C.; Pérez-Alfocea, F.; Hernandez, T.; Garcia, C.; Aguiar, N.O.; Canellas, L.P. Root growth promotion by humic acids from composted and non-composted urban organic wastes. *Plant Soil* **2011**, *353*, 209–220. [CrossRef]
84. Rathor, P.; Upadhyay, P.; Ullah, A.; Gorim, L.Y.; Thilakarathna, M.S. Humic acid improves wheat growth by modulating auxin and cytokinin biosynthesis pathways. *AoB Plants* **2024**, *16*, plae018. [CrossRef] [PubMed]
85. Ertani, A.; Schiavon, M.; Altissimo, A.; Franceschi, C.; Nardi, S. Phenol-containing organic substances stimulate phenylpropanoid metabolism in *Zea mays*. *J. Plant Nutr. Soil Sci.* **2011**, *174*, 496–503. [CrossRef]
86. Scaglia, B.; Pognani, M.; Adani, F. Evaluation of hormone-like activity of the dissolved organic matter fraction (DOM) of compost and digestate. *Sci. Total Environ.* **2015**, *514*, 314–321. [CrossRef] [PubMed]
87. Scaglia, B.; Nunes, R.R.; Rezende, M.O.O.; Tambone, F.; Adani, F. Investigating organic molecules responsible of auxin-like activity of humic acid fraction extracted from vermicompost. *Sci. Total Environ.* **2016**, *562*, 289–295. [CrossRef] [PubMed]
88. Upadhyay, S.K.; Srivastava, A.K.; Rajput, V.D.; Chauhan, P.K.; Bhojiya, A.A.; Jain, D.; Chaubey, G.; Dwivedi, P.; Sharma, B.; Minkina, T. Root Exudates: Mechanistic Insight of plant growth promoting rhizobacteria for sustainable crop production. *Front. Microbiol.* **2022**, *13*, 916488. [CrossRef]

89. Ghosh, T.; Maherukh, K.; Chowni, S.; Pal, A.K. Influence of Humic Acid and Vermicompost on Vegetative and Flowering Performances of African Marigold cv. Seracole in Indo-Gangetic Plains of West Bengal. *Int. J. Plant Soil Sci.* **2022**, *34*, 68–75. [CrossRef]
90. Sharma, I.; Kashyap, S.; Agarwala, N. Biotic stress-induced changes in root exudation confer plant stress tolerance by altering rhizospheric microbial community. *Front. Plant Sci.* **2023**, *14*, 1132824. [CrossRef] [PubMed]
91. Garnica, M.; Baigorri, R.; Francisco, S.S.; Zamarreño, A.M.; Garcia-Mina, J.M. Humic acid alleviates FE chlorosis in graminaceous plants through coordinated FE-Dependent and FE-Independent mechanisms. *Front. Plant Sci.* **2022**, *13*, 803013. [CrossRef] [PubMed]
92. Khan, R.U.; Khan, M.Z.; Khan, A.; Saba, S.; Hussain, F.; Jan, I.U. Effect of humic acid on growth and crop nutrient status of wheat on two different soils. *J. Plant Nutr.* **2018**, *41*, 453–460. [CrossRef]
93. López-Millán, A.F.; Duy, D.; Philippar, K. Chloroplast iron Transport proteins—Function and Impact on plant physiology. *Front. Plant Sci.* **2016**, *7*, 178. [CrossRef]
94. Jindo, K.; Canellas, L.P.; Albacete, A.; Figueiredo dos Santos, L.; Frinhani Rocha, R.L.; Carvalho Baia, D.; Oliveira Aguiar Canellas, N.; Goron, T.L.; Olivares, F.L. Interaction between Humic Substances and Plant Hormones for Phosphorous Acquisition. *Agronomy* **2020**, *10*, 640. [CrossRef]
95. Nardi, S.; Schiavon, M.; Muscolo, A.; Pizzeghello, D.; Ertani, A.; Canellas, L.P.; Garcia-Mina, J.M. Editorial: Molecular characterization of Humic Substances and regulatory processes activated in plants, volume II. *Front. Plant Sci.* **2024**, *15*, 1413829. [CrossRef] [PubMed]
96. Pantoja-Guerra, M.; Valero-Valero, N.; Ramirez, C.A. The auxin-like effect of substrate and auxin sensitivity of plant modulate the PGPR activity of *Lysinibacillus pinottii* sp. nov. PB211. *Chem. Biol. Technol. Agric.* **2024**, *11*, 165. [CrossRef]
97. Wang, Y.; Lu, Y.; Wang, L.; Song, G.; Ni, L.; Xu, M.; Nie, C.; Li, B.; Bai, Y. Analysis of the molecular composition of Humic Substances and their effects on physiological metabolism in maize based on untargeted metabolomics. *Front. Plant Sci.* **2023**, *14*, 1122621. [CrossRef] [PubMed]
98. Yildirim, E.; Ekinci, M.; Turan, M.; Ağar, G.; Dursun, A.; Kul, R.; Alim, Z.; Argin, S. Humic + Fulvic acid mitigated Cd adverse effects on plant growth, physiology and biochemical properties of garden cress. *Sci. Rep.* **2021**, *11*, 8040. [CrossRef]
99. Muscolo, A.; Sidari, M.; Cozzolino, V.; Nuzzo, A.; Nardi, S.; Piccolo, A. Molecular characteristics of Humic Substances from different origins and their effects on growth and metabolism of *Pinus laricio callus*. *Chem. Biol. Technol. Agric.* **2022**, *9*, 72. [CrossRef]
100. De Moura, O.V.T.; Berbara, R.L.L.; De Oliveira Torchia, D.F.; Da Silva, H.F.O.; Van Tol De Castro, T.A.; Tavares, O.C.H.; Rodrigues, N.F.; Zonta, E.; Santos, L.A.; García, A.C. Humic foliar application as sustainable technology for improving the growth, yield, and abiotic stress protection of agricultural crops. A review. *J. Saudi Soc. Agric. Sci.* **2023**, *22*, 493–513. [CrossRef]
101. Jan, J.A.; Nabi, G.; Khan, M.; Ahmad, S.; Shah, P.S.; Hussain, S.; Sehrish, S. Foliar Application of Humic Acid Improves Growth and Yield of Chilli (*Capsicum annum* L.) Varieties. *Pak. J. Agric. Res.* **2020**, *33*, 461. [CrossRef]
102. Yang, M.-H.; Zhang, L.; Xu, S.-T.; McLaughlin, N.B.; Liu, J.-H. Effect of water soluble humic acid applied to potato foliage on plant growth, photosynthesis characteristics and fresh tuber yield under different water deficits. *Sci. Rep.* **2020**, *10*, 7854. [CrossRef]
103. Wadas, W.; Dziugieł, T. Changes in Assimilation Area and Chlorophyll Content of Very Early Potato (*Solanum tuberosum* L.) Cultivars as Influenced by Biostimulants. *Agronomy* **2020**, *10*, 387. [CrossRef]
104. Ferrara, G.; Brunetti, G. Effects of the times of application of a soil humic acid on berry quality of table grape (*Vitis vinifera* L.) cv Italia. *Span. J. Agric. Res.* **2010**, *8*, 817–822. [CrossRef]
105. El-Tahlawy, Y.A.; Ali, O.A.M. Role of Humic Substances on Growth and Yield of Crop plant. In *Biostimulants for Crop Production and Sustainable Agriculture*; CABI: Wallingford, UK, 2022; pp. 159–178. [CrossRef]
106. Chen, Y.; Clapp, C.E.; Magen, H. Mechanisms of plant growth stimulation by Humic Substances: The role of organo-iron complexes. *Soil Sci. Plant Nutr.* **2004**, *50*, 1089–1095. [CrossRef]
107. Ampong, K.; Thilakaranthna, M.S.; Gorim, L.Y. Understanding the role of humic acids on crop performance and soil health. *Front. Agron.* **2022**, *4*, 848621. [CrossRef]
108. Hu, Z.-T.; Huo, W.; Chen, Y.; Zhang, Q.; Hu, M.; Zheng, W.; Shao, Y.; Pan, Z.; Li, X.; Zhao, J. Humic Substances derived from biomass waste during aerobic composting and hydrothermal treatment: A review. *Front. Bioeng. Biotechnol.* **2022**, *10*, 878686. [CrossRef]
109. Lanno, M.; Klavins, M.; Purmalis, O.; Shanskiy, M.; Kisand, A.; Kriipsalu, M. Properties of Humic Substances in Composts Comprised of Different Organic Source Material. *Agriculture* **2022**, *12*, 1797. [CrossRef]
110. Kulikova, Y.; Klementev, S.; Sirotkin, A.; Mokrushin, I.; Bassyouni, M.; Elhenawy, Y.; ElHadek, M.A.; Babich, O. Aqueous phase from hydrothermal liquefaction: Composition and toxicity assessment. *Water* **2023**, *15*, 168. [CrossRef]
111. Roulia, M. Humic S:ubstances a novel Eco-Friendly fertilizer. *Agronomy* **2022**, *12*, 754. [CrossRef]
112. Obeng, A.S.; Dunne, J.; Giltrap, M.; Tian, F. Soil organic matter carbon chemistry signatures, hydrophobicity and humification index following land use change in temperate peat soils. *Heliyon* **2023**, *9*, e19347. [CrossRef]

113. Pratiwi, G.R.; Nurrahma, A.H.I. Biochar and humic Substances Roles for nitrogen transformation in agriculture. *J. Tek. Pertan. Lampung (J. Agric. Eng.)* **2024**, *13*, 1442. [CrossRef]
114. Rahim, H.U.; Allevato, E.; Vaccari, F.P.; Stazi, S.R. Biochar aged or combined with humic substances: Fabrication and implications for sustainable agriculture and environment—A review. *J. Soils Sediments* **2023**, *24*, 139–162. [CrossRef]
115. Wiggins, Z.; Nandwani, D.; Akaeze, O.; Hui, D. Influence of Humic Substances and biochar on the yield of cantaloupe (*Cucumis melo* L.) in organic management systems. *Technol. Hortic.* **2023**, *3*, 7. [CrossRef]
116. Ji, R.; Liu, C.; Xu, Q.; Zhang, Y.; Chen, M.; Zhang, L.; Hu, F. Effect of Artificial Humic Acids Derived from Municipal Sludge on Plant Growth, Soil Fertility, and Dissolved Organic Matter. *Agriculture* **2024**, *14*, 1946. [CrossRef]
117. Rai, N.; Rai, S.P.; Sarma, B.K. Prospects for abiotic stress tolerance in crops utilizing Phyto- and Bio-Stimulants. *Front. Sustain. Food Syst.* **2021**, *5*, 754853. [CrossRef]
118. Ouni, Y.; Ghnaya, T.; Montemurro, F.; Abdelly, C.; Lakhdar, A. The role of Humic Substances in mitigating the harmful effects of soil salinity and improve plant productivity. *Int. J. Plant Prod.* **2014**, *8*, 353–374.
119. Burlakovs, J.; Kļaviņš, M.; Osinska, L.; Purmalis, O. The impact of humic substances as remediation agents to the speciation forms of metals in soil. *APCBEE Procedia* **2013**, *5*, 192–196. [CrossRef]
120. Peng, X.-X.; Gai, S.; Cheng, K.; Yang, F. Roles of humic substances redox activity on environmental remediation. *J. Hazard. Mater.* **2022**, *435*, 129070. [CrossRef]
121. Lipczynska-Kochany, E. Humic substances, their microbial interactions and effects on biological transformations of organic pollutants in water and soil: A review. *Chemosphere* **2018**, *202*, 420–437. [CrossRef] [PubMed]
122. Senko, O.; Maslova, O.; Stepanov, N.; Aslanli, A.; Lyagin, I.; Efremenko, E. Role of Humic Substances in the (Bio)Degradation of Synthetic Polymers under Environmental Conditions. *Microorganisms* **2024**, *12*, 2024. [CrossRef] [PubMed]
123. Xiao, K.; Horn, H.; Abbt-Braun, G. A review on the recovery of humic substances from anaerobic digestate of sludge as a potential fertilizer: Quantification, efficiency and interaction with pollutants. *Environ. Res.* **2024**, *267*, 120710. [CrossRef]
124. Sajjad, M.; Huang, Q.; Khan, S.; Khan, M.A.; Liu, Y.; Wang, J.; Lian, F.; Wang, Q.; Guo, G. Microplastics in the soil environment: A critical review. *Environ. Technol. Innov.* **2022**, *27*, 102408. [CrossRef]
125. Klemeš, J.J.; Van Fan, Y.; Tan, R.R.; Jiang, P. Minimising the present and future plastic waste, energy and environmental footprints related to COVID-19. *Renew. Sustain. Energy Rev.* **2020**, *127*, 109883. [CrossRef] [PubMed]
126. Piccolo, A.; Spaccini, R.; De Martino, A.; Scognamiglio, F.; Di Meo, V. Soil washing with solutions of Humic Substances from manure compost removes heavy metal contaminants as a function of humic molecular composition. *Chemosphere* **2019**, *225*, 150–156. [CrossRef] [PubMed]
127. Wang, Y.; Li, J.; Xu, L.; Wu, D.; Li, Q.; Ai, Y.; Liu, W.; Li, D.; Zhou, Y.; Zhang, B.; et al. EDTA functionalized Mg/Al hydroxides modified biochar for Pb(II) and Cd(II) removal: Adsorption performance and mechanism. *Sep. Purif. Technol.* **2024**, *335*, 126199. [CrossRef]
128. Tahat, M.M.; Alananbeh, K.M.; Othman, Y.A.; Leskovar, D.I. Soil Health and Sustainable Agriculture. *Sustainability* **2020**, *12*, 4859. [CrossRef]
129. Lal, R. Soil carbon sequestration impacts on global climate change and food security. *Science* **2004**, *304*, 1623–1627. [CrossRef] [PubMed]
130. Smith, H.B.; Vaughan, N.E.; Forster, J. Long-term national climate strategies bet on forests and soils to reach net-zero. *Commun. Earth Environ.* **2022**, *3*, 305. [CrossRef]
131. Gabriel, M.; Schöggl, J.-P.; Posch, A. Early Front-End Innovation Decisions for Self-Organized Industrial Symbiosis Dynamics—A Case Study on lignin Utilization. *Sustainability* **2017**, *9*, 515. [CrossRef]
132. Adamczyk, B.; Sietiö, O.M.; Straková, P.; Prommer, J.; Wild, B.; Hagner, M.; Pihlatie, M.; Fritze, H.; Richter, A.; Heinonsalo, J. Plant roots increase bothdecomposition and stable organic matter formation in borealforest soil. *Nat. Commun.* **2019**, *10*, 3982. [CrossRef] [PubMed]
133. Olaetxea, M.; De Hita, D.; Garcia, C.A.; Fuentes, M.; Baigorri, R.; Mora, V.; Garnica, M.; Urrutia, O.; Erro, J.; Zamarreño, A.M.; et al. Hypothetical framework integrating the main mechanisms involved in the promoting action of rhizospheric Humic Substances on plant root- and shoot- growth. *Appl. Soil Ecol.* **2017**, *123*, 521–537. [CrossRef]
134. Tang, C.; Li, Y.; Song, J.; Antonietti, M.; Yang, F. Artificial humic substances improve microbial activity for binding CO2. *iScience* **2021**, *24*, 102647. [CrossRef] [PubMed]
135. Spietz, T.; Kazankapova, M.; Dobras, S.; Kassenova, Z.; Yermagambet, B.; Khalimon, A.Y.; Stelmach, S. Characterization of Humic Acid Salts and Their Use for CO2 Reduction. *Minerals* **2024**, *14*, 947. [CrossRef]
136. Weber, J.; Chen, Y.; Jamroz, E.; Miano, T. Preface: Humic Substances in the Environment. *J. Soils Sediments* **2018**, *18*, 2665–2667. [CrossRef]
137. Huang, J.; Xu, P.; Shao, M.; Wei, B.; Zhang, C.; Zhang, J. Humic acids alleviate dextran sulfate sodium-induced colitis by positively modulating gut microbiota. *Front. Microbiol.* **2023**, *14*, 1147110. [CrossRef] [PubMed]

138. Bi, D.; Yuan, G.; Wei, J.; Xiao, L.; Feng, L.; Meng, F.; Wang, J. A Soluble Humic Substance for the Simultaneous Removal of Cadmium and Arsenic from Contaminated Soils. *Int. J. Environ. Res. Public Health* **2019**, *16*, 4999. [CrossRef] [PubMed]
139. Holatko, J.; Hammerschmiedt, T.; Kucerik, J.; Kintl, A.; Baltazar, T.; Malicek, O.; Latal, O.; Brtnicky, M. Fertilisation of permanent grasslands with digestate and its effect on soil properties and sustainable biomass production. *Eur. J. Agron.* **2023**, *149*, 126914. [CrossRef]
140. Fu, B.; Yu, C.; Sun, J.; Wang, H.; Adachi, Y. The influence of Humic Substances on environmental behavior of antibiotic resistance genes: A mini-review. *J. Hazard. Mater. Adv.* **2022**, *8*, 100173. [CrossRef]
141. Ma, Y.; Cheng, X.; Zhang, Y. The Impact of Humic Acid Fertilizers on Crop Yield and Nitrogen Use Efficiency: A Meta-Analysis. *Agronomy* **2024**, *14*, 2763. [CrossRef]
142. Raina, N.; Zavalloni, M.; Viaggi, D. Incentive mechanisms of carbon farming contracts: A systematic mapping study. *J. Environ. Manag.* **2024**, *352*, 120126. [CrossRef]
143. Billingham, K.L. Humic Products–Potential or Presumption for Agriculture. Do Humic Products Have a Place in Australian Grazing Enterprises? *IGC Proc.* **2020**, *3*. Available online: https://uknowledge.uky.edu/igc/22/2-11/3 (accessed on 28 January 2025).
144. Grover, D.; Mishra, A.K.; Rani, P.; Kalonia, N.; Chaudhary, A.; Sharma, S. Soil management in Sustainable agriculture: Principles and techniques. In *Technological Approaches for Climate Smart Agriculture*; Springer: Cham, Switzerland, 2024; pp. 41–77. [CrossRef]
145. Meddich, A. Biostimulants for Resilient Agriculture—Improving Plant Tolerance to Abiotic Stress: A Concise Review. *Gesunde Pflanz.* **2023**, *75*, 709–727. [CrossRef]
146. Wreford, A.; Ignaciuk, A.; Gruère, G. *Overcoming Barriers to the Adoption of Climate-Friendly Practices in Agriculture*; OECD Food, Agriculture and Fisheries Working Papers; No. 101; OECD Publishing: Paris, France, 2017. [CrossRef]
147. Chin, Y.-P.; McKnight, D.M.; D'Andrilli, J.; Brooks, N.; Cawley, K.; Guerard, J.; Perdue, E.M.; Stedmon, C.A.; Tratnyek, P.G.; Westerhoff, P.; et al. Identification of next-generation International Humic Substances Society reference materials for advancing the understanding of the role of natural organic matter in the Anthropocene. *Aquat. Sci.* **2023**, *85*, 32. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Chapter 3 - Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli

Angela Maffia [1], Federica Marra [1], Santo Battaglia [1], Carmelo Mallamaci [1], **Mariateresa Oliva** [1], Carmelo Mallamaci [1] and Adele Muscolo [1*]

[1] Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy.

*Correspondence: amuscolo@unirc.it

Soil Systems

https://doi.org/10.3390/soilsystems8020053

Received: 9 April 2024

Revised: 6 May 2024

Accepted: 9 May 2024

Published: 15 May 2024

soil systems

MDPI

Article

Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli

Angela Maffia, Federica Marra, Santo Battaglia, Mariateresa Oliva, Carmelo Mallamaci and Adele Muscolo *

Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); federica.marra@unirc.it (F.M.); battaglia.santo96@gmail.com (S.B.); mariateresa.oliva@unirc.it (M.O.); carmelo.mallamaci@unirc.it (C.M.)

* Correspondence: amuscolo@unirc.it; Tel.: +39-09651694364

Abstract: In this work, environmentally sound technologies for converting organic wastes into fertilizers to improve soil sustainability and crop yield have been identified and assessed. Wet wastes were combined with 50% wood sawdust and 50% wet wastes (Compost 1) or (10% Straw + 90% wet wastes) (Compost 2) to produce soil improvers with a balanced level of nutrients, and their effectiveness on soil ecosystem functioning have been tested and compared to horse manure (HM) and nitrogen–phosphorous–potassium (NPK) fertilizers. Unfertilized soil was used as a control. Soil chemical and biological properties have been detected after the harvesting of broccoli and red cabbage (90 days from the initial treatments). Three independent experiments have been conducted in an open field in a randomized complete block design with three replications (n = 9). The results showed that Compost 1 had the highest C/N ratio and cation exchange capacity (CEC), indicating a better humification of the wet material. Compost 1, even if it contained a minor amount of organic carbon, as well as less activity of fluorescein diacetate (FDA) and dehydrogenase (DHA) than Compost 2, was the most effective in improving soil quality, significantly increasing the labile fraction of organic matter, the oxidative enzyme (DHA), microbial biomass, and crop yield. Both composts increased crop productivity.

Keywords: waste compost; soil fertility; broccoli calabrese; red cabbage; soil amendments

check for updates

Citation: Maffia, A.; Marra, F.; Battaglia, S.; Oliva, M.; Mallamaci, C.; Muscolo, A. Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli. *Soil Syst.* **2024**, *8*, 53. https://doi.org/10.3390/soilsystems8020053

Academic Editors: Douglas Guelfi, Heitor Cantarella and Flávio Henrique Silveira Rabêlo

Received: 9 April 2024
Revised: 6 May 2024
Accepted: 9 May 2024
Published: 15 May 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

In the face of global challenges, such as population growth, climate change, and diminishing agricultural resources, there is an increasing imperative to develop sustainable agricultural practices that simultaneously enhance soil fertility, mitigate waste-disposal issues, and reduce environmental impacts [1]. Based on the latest UN projections, the world's population may rise to roughly 8.5 billion by 2030, 9.7 billion by 2050, and could peak around 10.4 billion in the 2080s. Consequently, our yearly food supply needs to sustainably meet the demands of this growing populace [2]. Sustainable agricultural methods offer ways to produce food and other agricultural products with minimal environmental impact. This ensures consistent food access and availability and environmental and human health safeguards. Sustainable agriculture is linked to food security, which encompasses consistent food availability; adequate production; affordability; sufficient nutrition in terms of energy, proteins, and micronutrients; safety; and the economic stability to maintain these factors [3]. It is imperative to identify and analyze well-established approaches aimed at fostering sustainable agriculture, many of which prioritize ecosystem health.

These approaches are characterized by clear principles and encompass environmental, economic, and social objectives. They have evolved as methodological strategies over time, like agroecology and sustainable intensification, or were prioritized from the outset, such as carbon farming.

Composting represents a promising solution, offering a means to recycle organic materials while generating nutrient-rich soil amendments [4]. In essence, composts derived from waste management within the context of the circular economy, such as biowaste, organic waste, and green waste, are considered acceptable for use in organic agriculture under Regulation EU 2021/1165 [5].

This allowance is contingent upon these composts originating from a recognized separate collection system within the respective EU member state and adhering to specified limits for heavy metal content. Compost, when used as a fertilizer or soil conditioner, can significantly enhance soil quality [6,7]. It accomplishes this by improving aeration, optimizing water content, enhancing aggregate stability, and thereby bolstering resistance against erosion. Furthermore, compost enriches the soil with both macro and micronutrients, fostering healthier plant growth, and augments the cation exchange capacity, as demonstrated by Muscolo et al. (2018) [6] and Ghimire et al. (2023) [8]. Activating the soil microbiota and increasing its biomass are additional benefits, although the extent of these effects relies heavily on the quality and quantity of organic matter, as observed by Bonanomi et al. (2020) [9] and Sunman et al. (2022) [10]. When it comes to the risk of nitrate leaching, composted organic waste is generally considered to pose minimal risk [11].

This manuscript explores the scientific dimensions of composting through the lens of a specific approach: the utilization of wood sawdust and vegetable wastes as composting materials.

The selection of wood sawdust and vegetable wastes for composting is rooted in their unique compositional characteristics. Wood sawdust, a by-product of various woodworking processes, is recognized for its high carbon content and lignocellulosic structure [12]. This provides an excellent source of carbon, crucial for establishing an optimal carbon-to-nitrogen ratio in the composting process. Additionally, wood sawdust represents to the wood industry a waste to be disposed of with economic implications. In contrast, vegetable wastes, including kitchen scraps and garden trimmings, contribute nitrogen-rich organic matter. When these materials are co-composted, they hold the potential to create a well-balanced mixture, essential for the efficient decomposition of organic matter [13].

The science of composting hinges upon the microbial-driven biological transformation of organic materials into stabilized organic matter known as humus. This process involves a complex interplay of organisms, including bacteria, fungi, actinomycetes, and earthworms that break down the organic compounds present in the feedstock. In the case of wood sawdust and vegetable-waste composting, the intricate lignocellulosic structure of sawdust provides an intriguing substrate for microbial colonization and degradation, leading to the release of carbon and other nutrients [14].

The resulting compost, characterized by a dark, crumbly texture, not only sequesters carbon but also embodies essential nutrients such as nitrogen, phosphorus, and potassium, as well as micronutrients required for plant growth. Beyond its nutrient content, the compost enhances soil structure, moisture retention, and microbial diversity, ultimately fostering improved soil health and agricultural productivity. Furthermore, this manuscript a part to delve into the scientific aspects of composting management, addressing critical factors such as temperature dynamics, aeration, moisture content, and composting timeframes, explore the effects of compost as fertilizer on broccoli and cabbage growth and yield. Particularly, the growth parameters related to productivity and the parameters related to plant performance have been detected and discussed. This manuscript explores the scientific intricacies of this composting method, shedding light on its potential to transform wastes into a valuable resource, mitigate greenhouse-gas emissions, and enhance soil fertility and crop yield, and provides a compelling avenue toward a more sustainable and resilient agricultural future.

2. Materials and Methods

2.1. Feeding Materials

The raw organic materials employed for composting comprised a variety of components and precise vegetable wastes (like rocket salad, lettuce, cabbage, carrots, and valerian).

The two composts have been prepared using different percentages of the vegetable residues (Table 1).

Table 1. Compostable raw materials of different composts used.

Compost ID	Compostable Raw Material
Compost 1 (C1)	50% wood sawdust + 50% wet waste, such as kitchen and restaurant scraps.
Compost 2 (C2)	10% Straw + 90% wet wastes, such as kitchen and restaurant scraps.

2.2. Composting Process Setup

Sawdust and vegetable residues, as well as straw and vegetable residues, were carefully deposited into dedicated electric composters and subjected to the composting process. The electric composter contains multiple chambers for a better composting process. The waste in the second chamber does not come into contact with the fresh waste; the temperatures of the two chambers are managed independently for the better development of microbial populations. The tank dimensions were 350 cm × 100 cm × 131 cm and had a capacity of 10 tons/year. This composting protocol was replicated three times for each compost mixture. The composting conditions were controlled as follows: an initial mesophilic phase for 8 days at 29 °C followed by a thermophilic phase lasting 20 days at 50 °C.

A second mesophilic phase extended for 92 days at 27 °C. The temperature increases during the composting process resulted from the robust microbial activity, facilitated by efficient ventilation within the mixture.

The ventilation process is maintained and accelerated by the continuous supply of air and the continuous movement of the organic material. This ventilation process guaranteed the presence of ample oxygen levels, thereby promoting biological processes while maintaining optimal aerobic conditions, as documented by Liang et al. (2003) [15]. Following this mesophilic phase, a stabilization transitioning phase with a stable temperature of 20 °C for 30 days was settled to stabilize the compost until the conclusion of the composting cycle. This stability was attributed to reduced microbial activity and a diminishing quantity of organic substrate available for decomposition. The moisture content was upheld at 50%, and the oxygen percentage consistently exceeded 15%. Temperature, moisture, and oxygen levels were monitored daily using a probe strategically placed in the center of the composting mass, ensuring they remained within the predefined parameters. Water was added as needed to sustain the 50% moisture level. Daily agitation of the mixtures was performed to guarantee oxygen levels above 15%, thereby promoting the aerobic decomposition of organic matter into stable humus. Comprehensive decomposition and stabilization of the materials were accomplished over a span of 4 months. Subsequently, all compost batches underwent an air-drying process, were finely crushed to pass through a 2 mm sieve, and underwent thorough blending to ensure uniformity.

2.3. Chemical Characterization of Composts

The chemical analysis of the composts was conducted in accordance with the protocols outlined in the ANPA manual from 2001 [16]. To evaluate the rate of organic matter mineralization, the reduction in organic matter content over time was assessed by using the following equation (Equation (1)):

$$\text{Organic matter loss (\%)} = [(\text{Initial mass of carbon} - \text{Final mass of carbon})/\text{Initial mass of carbon}] \times 100 \quad (1)$$

The determination of fluorescein 3,6-diacetate hydrolase activity followed the procedure established by Adam and Duncan [17]. The results were expressed as mg fluorescein released per gram of dry soil, following Perucci et al. (1992) [18].

Dehydrogenase (DHA) activity was determined as outlined by von Mersi and Schinner (1991) [19]. The absorbance of the soil filtrate was measured at 490 nm.

Water-soluble phenols (WSP) were detected by extracting the soil with water and determining their concentration using the Folin–Ciocalteau reagent, following Box's method from 1983 [20]. The cation exchange capacity (CEC) was determined using an aqueous solution of $BaCl_2$ buffered to pH 7.0 to saturate the soil-exchange complex, following Hendershot and Duquette (1986) [21].

Compost maturity was assessed by employing cucumber (*Cucumis sativus* L.) seeds, following the method described by Gariglio et al. (2002) [22]. The Germination Index (GI), which combines measures of relative seed germination (%) and relative root elongation (%), was used to evaluate compost toxicity. This method is particularly sensitive and capable of detecting both low-level and high-level toxicity affecting root growth and germination, respectively. A GI value higher than 60% indicates the non-phytotoxicity of the compost, as established by Zucconi et al. (1981) [23].

The organic matter mineralization rate was assessed by evaluating the loss of organic matter over time, Organic matter loss was calculated following Equation (1).

The absorption capacity of the composts, in relation to Na^+ and Cl^- ions, has been calculated using the following Formula (2):

$$AC = (Ci - Cf)\ m \times VAC = m(Ci - Cf) \times V \quad (2)$$

where:

- AC is absorption capacity;
- Ci is the initial concentration of ions;
- Cf is the final concentration of ions;
- m is the compost weight;
- V is the volume of the solution (40 mL).

2.4. Soil Characteristics and Treatments

The experiment was carried out in sandy-loam soil belonging to Cambisol (WRB, 2022) [24], located in Motta San Giovanni, Loc. Liso, Italy (37.9991° N. 15.6999° E).

The average temperature of the coldest months, January and February, stands at +11.9 °C; that of the hottest month, August, is +26.1 °C. The average annual precipitation is around 493 mm, with a minimum in summer and a peak in autumn–winter. The fertilization experiments consisted of three replicate plots for each condition. Each plot measured 18 m^2 and was set up using a single-factor randomized complete block design. The soil received a fertilization treatment using the two composts distributed at a depth of 10/15 cm. In each designated plot, composts were incorporated based on the organic matter content precisely at rates of 3.1 Mg/ha for composts, horse manure (HM, 4.3 Mg/ha), and NPK (20:10:10) at 1.7 Mg/ha. To maintain consistent moisture levels, the plants were regularly irrigated to ensure a water content of 70% of field capacity across all parcels. The experiment was replicated three times. Two different crops have been used to test the effectiveness of the two produced composts, namely ramous Calabria broccoli and red cabbage.

The differently treated crops were collected when they reached ripeness level, based on visual characteristics such as size, shape, and color. Cabbage cultivated with Compost 1 matured in a range of 78 days, while those grown with Compost 2 maturated in 85 days, with HM in 88 days, and with NPK taking 90 days to mature. Broccoli was ready to be harvested 70 days after transplanting when cultivated with Compost 1, 79 days when cultivated with Compost 2, 83 days when grown with NPK, and 80 days with HM. Within each plot, for both crops (broccoli and cabbage), 3–4 plants/m^2 were planted for each treatment. The spacing between individual plants was set at 40 cm, with 60 cm between rows. Throughout the experiment, the parcels were irrigated to maintain the soil moisture at 70% of field capacity. Soil humidity was continuously monitored using a direct-read soil pH/moisture meter—R181 to ensure consistent soil moisture levels in both soil types.

2.5. Soil Analysis

Soils were collected in each parcel at the end of the experiment (90 days), as reported below for the specific crop species and fertilization used. Soils have been air-dried and sieved through a 2 mm sieve for chemical analyses, while fresh soil sieved to 2 mm was used for microbiological analyses. The soil water content was expressed in percentage. The water content was determined at the beginning of the experiment and every 15 days during the entire experiment for all soil treatments. Particle-size analysis was carried out by using the method of Bouyoucos et al. (1962) [25]. Dry matter (dm) was determined by weighing the samples after 24 h at 105 °C; pH and EC were measured as reported in Muscolo et al. (2017) [26]. Organic carbon was determined by oxidimetric method following the Walkley–Black procedure [27]; total nitrogen was determined by the digestion procedure, using sulfuric acid at temperatures of 380 °C following the method of Kjeldahl et al. (1883) [28]. The amount of microbial biomass carbon (MBC) was determined by using the chloroform fumigation extraction procedure [29] with field-moist samples (equivalent to 20 g dry wt.). The filtered soil extracts of both fumigated and unfumigated samples were analyzed for soluble organic C using the methods of Walkley and Black [27]. Microbial biomass C was estimated on the basis of the differences between the organic C extracted from the fumigated soil and that from the unfumigated soil, and an extraction efficiency coefficient of 0.38 was used to convert soluble C into biomass C [29]. The microbial population was extracted following the method of Insam and Goberna [30]. Two grams of soil and 30 glass beads were mixed with 20 mL 0.90% NaCl and shaken for 20 min at 20 °C, then centrifuged at 4 °C for 1 h at 12,000× *g* to separate the bacteria from the solid particles. The supernatant was used for further dilutions with a sterile one-fourth-strength Ringer solution so as to standardize the inoculum density. The soil's bacterial population was estimated by Waksman et al. (1952) [31] with method using the nutrient agar medium at 10^5 dilutions. The fungal population was estimated by the dilution-plate method using Martin's Rose Bengal agar medium at 10^3 dilutions in water [32]. The activities of fluorescein 3,6-diacetate hydrolase (FDA), and dehydrogenase (DHA), as well as the water-soluble phenol amount, ion concentrations, and cationic exchange capacity (CEC), were determined as reported in Section 2.3. Three soil samples for each crop and each specific fertilization have been collected. All the analyses were performed in triplicates. Thus, for each cultivar and condition, n = 9.

2.6. Crop Growth Assessment

Each cultivar was analyzed for the following growth parameters: plant height (PH) from the soil level to the highest point of the plant, leaf area (LA, cm^2), leaf length (LL, cm), leaf width (LW, cm), leaf humidity (LH, %) from the basal leaves to the last open leaf, fruit-head diameter (HD, cm) and yield (tons/hectare). For the estimation of total chlorophyll content, 100 mg of leaf tissue were finely ground in liquid nitrogen and suspended in dimethyl sulfoxide (DMSO). The suspension was maintained at 65 °C for 30 min. The final volume was adjusted to 10 mL with DMSO, and the absorbance was recorded at 645 and 663 nm. The total chlorophyll content was calculated as reported by Hiscox et al. (1979) [33].

2.7. Mineral Assay

Cations (Na, K, Ca, Mg) were extracted from samples and analyzed using ion chromatography (DIONEX ICS-1100, Thermo Fisher Scientific, Waltham, MA, USA). One g of dry material was ashes at 550 °C for 6 h in a porcelain capsule. The ash was then acidified for 30 min at 100 °C using 1M of HCl solution (10 mL). Finally, it was filtered using Whatman 1 and measured using the ion chromatograph with 20 mM methane–sulfonic acid as an eluent. The Fe concentration was determined using atomic absorption spectrophotometry (model 2380, PerkinElmer Co., Waltham, MA, USA). Four mixed standard solutions with concentrations of 1, 5, 25, and 50 ppm of each of the four desired anions were used to plot the calibration curve. The linear relationship between the peak area and concentration

was confirmed experimentally. The amount of each cation was calculated using its own standard curve. The amount of each anion was calculated using ion chromatography (DIONEX) and comparing the results with a multi-ion cation standard curve (Multi Ion Cation IC standard solution, Specpure®, Dionex, Thermo Fisher, Milan, Italy) [34].

All solvents and reagents were purchased from Panreac (Barcelona, Spain). The bioconcentration factor (cation or anion in root/cation or anion in soil), bioaccumulation coefficient (cation or anion in leaves/cation or anion in soil), and translocation factor (cation or anion in leaves/cation or anion in roots) were detected.

2.8. Chlorophyll Fluorescence Imaging

The photosynthetic efficiency of cabbage and broccoli leaves, differently fertilized, was evaluated by using an Imaging PAM Fluorometer (Walz). The chlorophyll fluorescence parameters detected were as follows: maximum quantum yield of PSII photochemistry (Fv/Fm); effective quantum yield of PSII photochemistry (Y(II)); quantum yield of regulated energy dissipation at PSII (Y(NPQ)); quantum yield of non-regulated energy dissipation at PSII (Y(NO)); non-photochemical quenching coefficient (NPQ), and electron transport rate (ETR). The maximum PSII quantum yield (Fv/Fm), photochemical fluorescence quenching (qP), non-photochemical fluorescence quenching (NPQ), and ETR have been evaluated and analyzed for the indication they give. Fv/Fm showed the maximum efficiency of energy conversion in PSII. qP indicates the rate of photochemical reactions in the chloroplast electron transport chain in vivo. NPQ indicates the amount of excess energy that was absorbed by chlorophyll but was not used by the electron transport chain and was converted to heat [35]. The ETR electron transport rate is proportional to the photosynthetic activity, and a higher value indicates higher carbon fixation activity. These parameters are measured in relative units.

2.9. Statistical Analyses

Data are expressed as the means of three analyses for each treatment and three analyses for different compost analyses. Significant difference tests were carried out to analyze the effects of fertilizers on each of the various parameters measured. Homogeneity of variance and normality were tested, respectively, with the tests by Bartlett and Shapiro with a p value of 0.05. For all other variables, a one-way ANOVA ($p < 0.05$) was performed, followed by Tukey's post hoc test tests, to find significant differences between treatments ($p < 0.05$). The ANOVA and t-test were carried out using XLStat. To explore the relationships among different fertilizers on the soil, broccoli, and red cabbage, we analyzed parameter datasets using principal component analysis (PCA) with XLStat.

3. Results

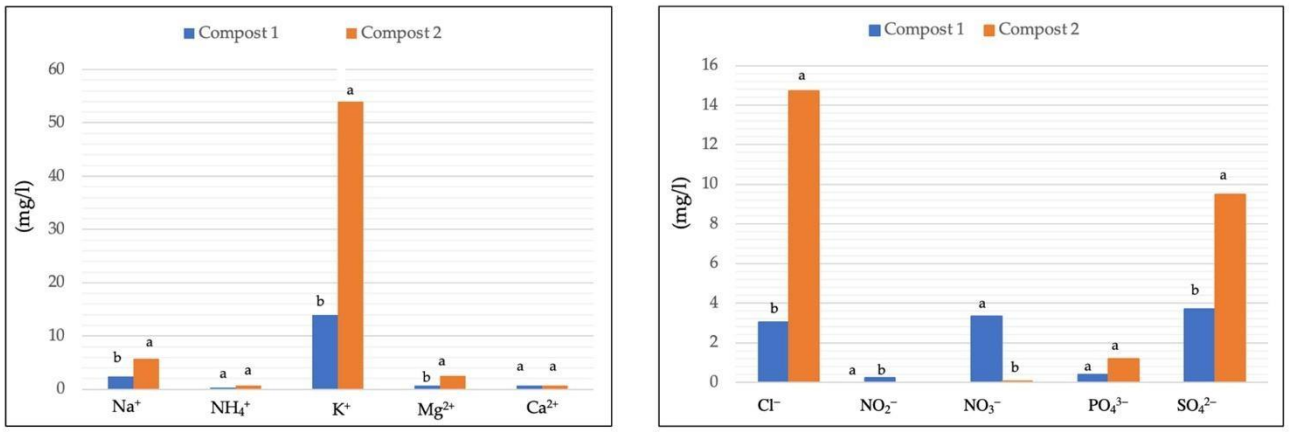
3.1. Compost Properties

The composting procedure underwent three repetitions through independent experiments. The outcomes consistently revealed that each compost derived from these experiments exhibited identical chemical characteristics. This observation strongly indicated that the adopted procedure has been successfully standardized, ensuring the reproducibility of the results over time. After a 4-month composting period, the analysis revealed noteworthy distinctions between the two composts obtained using the same methodology (Table 2). Both C1 and C2 composts displayed highly alkaline pH levels. C2 exhibited the highest total organic carbon and total nitrogen, while C1 and C2 differed in their C/N ratio (21.57 for C1 and 11.97 for C2). The $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio was the highest in C2, whereas the ON/TN ratio was significantly greater in C1 than in C2 (Table 2). Despite all composts being nutrient-rich (Figure 1a,b), C2 contained more nutrients than C1, in particular potassium and magnesium (Figure 1a), C1 contained the highest amount of NO_2 and NO_3. Conversely, C2 had the greatest amount of phosphates and sulfates (Figure 1b). Notably, C2 contained eight times more water-soluble phenols (WSP) and concurrently exhibited a greater cation exchange capacity and FDA and DHA activities than C1 (Figure 2). Assessing compost

maturity through phytotoxicity, as indicated by the germination index (Figure 3), revealed that C1 did not exhibit phytotoxicity in *Cucumis sativus*. The germination index measured 6 days post-germination at 25%, 50%, and 75% compost concentrations consistently exceeded 80%, classifying it as phytonutrient. These findings align with the overall germination index, ranging from 67.5% to 95%, confirming the non-phytotoxic nature of both composts.

Table 2. Physico-chemical properties of the two composts obtained from different raw materials Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process. pH (H_2O and KCl); electric conductivity (EC, mS cm^{-1}); water content (WC, %); total organic carbon (TOC, %); Total Nitrogen (TN, %); carbon–nitrogen ratio (C/N); ammonium-nitrogen–nitrate-nitrogen ratio (NH_4^+-N/NO_3^--N); organic nitrogen–total nitrogen ratio (ON/TN, %), water-soluble phenols (WSP µg GAE g^{-1} d.s). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences at $p \leq 0.05$.

Physico-Chemical Properties	COMPOST 1	COMPOST 2
pH_{H_2O}	9.05 ^{b} ± 0.1 very strongly alkaline	9.90 ^{a} ± 0.1 very strongly alkaline
pH_{KCl}	8.39 ^{b} ± 0.1	9.28 ^{a} ± 0.1
E.C.	5.01 ^{a} ± 0.12	5.06 ^{a} ± 0.11
Water content	56.8 ^{a} ± 2	45.9 ^{b} ± 1.5
TOC	16.8 ^{b} ± 0.9	24.0 ^{a} ± 1
TN (%)	0.78 ^{b} ± 0.05	2.0 ^{a} ± 0.1
C/N	21.57 ^{a} ± 1	11.97 ^{b} ± 0.9
NH_4^+-N/NO_3-N	1.30 ^{b} ± 0.3	2.80 ^{a} ± 0.2
ON/TN	90 ^{a} ± 2	60 ^{b} ± 1
WSP	0.90 ^{b} ± 0.05	7.03 ^{a} ± 0.3



(**a**) (**b**)

Figure 1. Cation concentration (mgL^{-1}) (**a**) and anion concentration (mgL^{-1}) (**b**) detected in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process at the end of the composting process. Different letters indicate significant differences (Turkey's test $p \leq 0.05$).

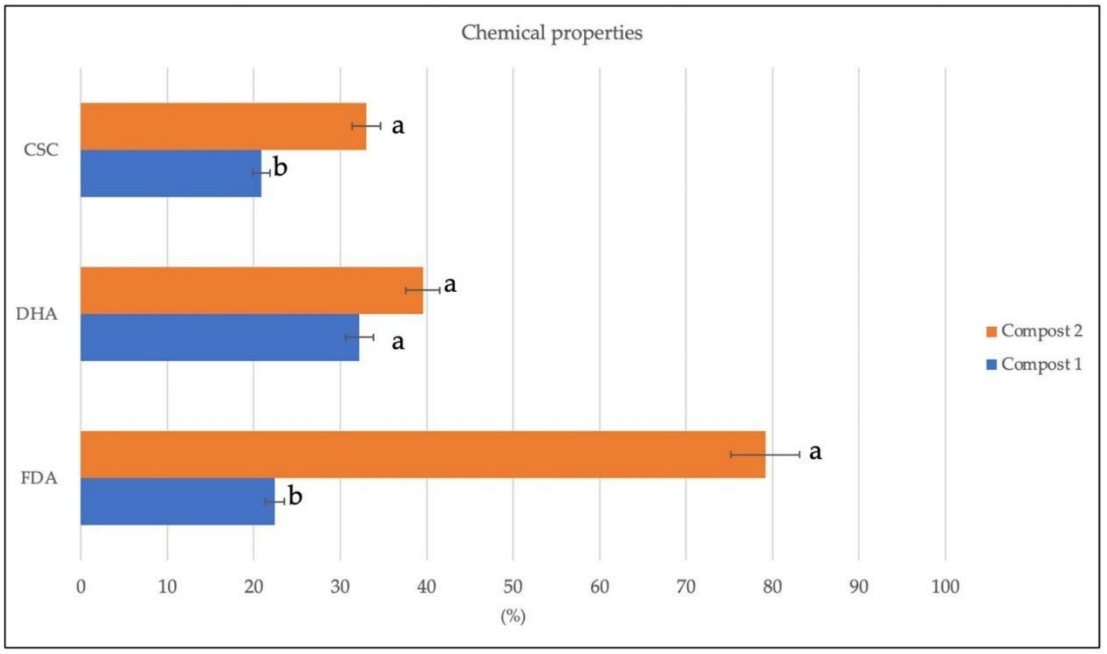


Figure 2. Fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.w.), dehydrogenase (DHA, µg TTF g^{-1} h^{-1}d.w.), cation exchange capacity (CEC, $cmol^{(+)}$ Kg^{-1}) detected in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process. Different letters indicate significant differences (Turkey's test $p \leq 0.05$).

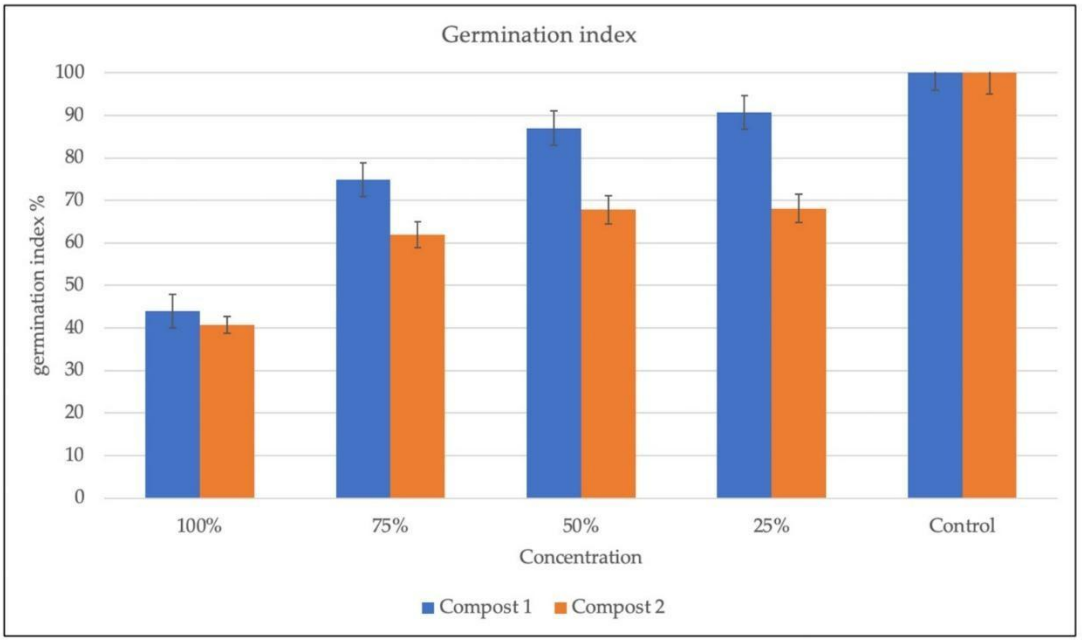


Figure 3. Germination index in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process.

In terms of adsorption capacity for composts, it was observed that all the composts exhibited the ability to adsorb both sodium and chloride, albeit to varying degrees. The phenomenon of negative values recorded at 0 mM of sodium and chloride can be explained by considering that, in the absence of the addition of these two ions in the solutions, other ions remain adsorbed in the compost-available surfaces. This process leads to the generation of negative adsorption values for sodium and chloride. Notably, C2 demonstrated the

highest sodium adsorption capacity, outperforming the other compost. Meanwhile, C1 exhibited an optimal sodium removal capacity at 50 mM NaCl, with a subsequent decline in efficiency as the sodium concentration increased (Table 3). Turning attention to chloride adsorption capacity, it was observed that all composts possessed the capability to remove chloride ions. As the chloride concentration increased, the adsorption capacity of all composts gradually intensified. Notably, C2 displayed the most significant adsorption capacity for chloride ions, further emphasizing its efficacy in the removal of both sodium and chloride.

Table 3. The data regarding the absorption capacity of the analyzed compost related to sodium and chloride. Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

	0 mM	25 mM	50 mM	100 mM	150 mM
ID	Na^+	Na^+	Na^+	Na^+	Na^+
C1	$-4.56^{e} \pm 0.15$	$8.09^{d} \pm 0.76$	$12.60^{c} \pm 0.23$	$53.58^{b} \pm 0.24$	$93.95^{a} \pm 1.4$
C2	$-3.26^{e} \pm 0.2$	$88.11^{d} \pm 0.6$	$109.51^{c} \pm 0.2$	$243.50^{a} \pm 0.4$	$212.88^{b} \pm 1.3$
ID	Cl^-	Cl^-	Cl^-	Cl^-	Cl^-
C1	$-5.86^{e} \pm 0.2$	$53.23^{d} \pm 0.16$	$120.59^{c} \pm 1.6$	$138.23^{b} \pm 1.8$	$367.99^{a} \pm 3.6$
C2	$-4.330^{e} \pm 0.1$	$87.40^{d} \pm 0.5$	$124.08^{c} \pm 0.1$	$311.51^{b} \pm 0.1$	$461.24^{a} \pm 0.2$

3.2. Soil Characteristics

Table 4 shows the analysis of the soil at time zero, before initiating the various fertilization treatments. It was an alkaline sandy-loam soil, with 2.37% organic matter, poor in anions and cations, and with a CEC of 13 $cmol^{(+)}\ kg^{-1}$. Bacteria were more abundant than fungi and actinomycetes, as also evidenced by a greater DHA with respect to FDA.

Table 4. Chemical and biochemical properties of soil located in Motta San Giovanni before fertilization. WC (water content %), pH $_{H_2O}$ in water and pH_{KCl} in potassium chloride; EC = electric conductivity (dS/m); WSP = water-soluble phenols (µg TAE g^{-1} ds): OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon–nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (µg C g^{-1} f.s.); Dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.s.), fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.s.), BACT (bacteria, UFC g^{-1} f.s.), FUN (fungi (UFC g^{-1} f.s.), ACT (Actinomycetes, UFC g^{-1} f.s.), CEC = cation exchange capacity ($cmol^{(+)}$ Kg^{-1} d.s.). Data are the means of three replicates ± standard deviation.

	SOIL
Skeleton (%)	45 ± 0.01
Sandy %	65 ± 0.02
Clay %	23 ± 0.12
Silt %	12 ± 0.23
Textural Class	Sandy-loam
WC	18 ± 0.4
pH (H_2O)	8.5 ± 0.32
pH (KCl)	7.8 ± 0.53
EC	307.3 ± 12.3
CEC ($cmol^{(+)}\ kg^{-1}$)	16 ± 1.7
OC	1.37 ± 0.13
TN	0.19 ± 0.14
C/N	7.21 ± 0.13
WSP	276.1 ± 4.5
MBC	376 ± 8.6

Table 4. *Cont.*

	SOIL
FDA	2.1 ± 0.12
DHA	15.11 ± 0.22
BACT	0.9×10^5
FUN	2.6×10^4
ACT	2.7×10^4
Na^+	0.117 ± 0.32
K^+	0.100 ± 0.26
Ca^{2+}	0.311 ± 0.06
Mg^{2+}	0.011 ± 0.16
Cl^-	0.222 ± 0.11
NO_2^-	nd
NO_3^-	nd
PO_4^{3-}	nd
SO_4^{2-}	0.134 ± 0.11

All the fertilizers used (both composts, NPK, and HM) affected the soil's chemical properties with respect to the control, except for texture, which remained unchanged.

The pH did not change with the treatments. Instead, the EC increased in a particular way with the additions of both composts and much more with C2, suggesting an addition of nutrients. Adding composts to the soil can provide a great quantity of nutrients in the form of hydrated salts, helping to increase the percentage of water in the soils.

The Pearson correlation coefficient also evidenced synergies between cations and among cations and anions. Shortly, potassium was correlated with calcium, suggesting a synergism among them and also with anions, in particular with sulfate (Tables 5 and 6).

Table 5. Soil ions and cations correlation matrix. Pearson values in bold are different from 0 with a significance level alpha = 0.05.

Variables	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Na^+	**1**	**0.908**	0.125	0.326	0.582	**0.998**	**0.998**	**0.998**	**0.931**
K^+	**0.908**	**1**	0.408	0.582	0.759	**0.893**	**0.893**	**0.893**	**0.980**
Ca^{2+}	0.125	0.408	**1**	**0.887**	0.793	0.090	0.090	0.090	0.441
Mg^{2+}	0.326	0.582	**0.887**	**1**	0.672	0.309	0.309	0.309	0.635
Cl^-	0.582	0.759	0.793	0.672	**1**	0.540	0.540	0.540	0.751
NO_2^-	**0.998**	**0.893**	0.090	0.309	0.540	**1**	1.000	**1.000**	**0.921**
NO_3^-	**0.998**	**0.893**	0.090	0.309	0.540	**1.000**	**1**	**1.000**	**0.921**
PO_4^{3-}	**0.998**	**0.893**	0.090	0.309	0.540	**1.000**	**1.000**	**1**	**0.921**
SO_4^{2-}	**0.931**	**0.980**	0.441	0.635	0.751	**0.921**	**0.921**	**0.921**	**1**

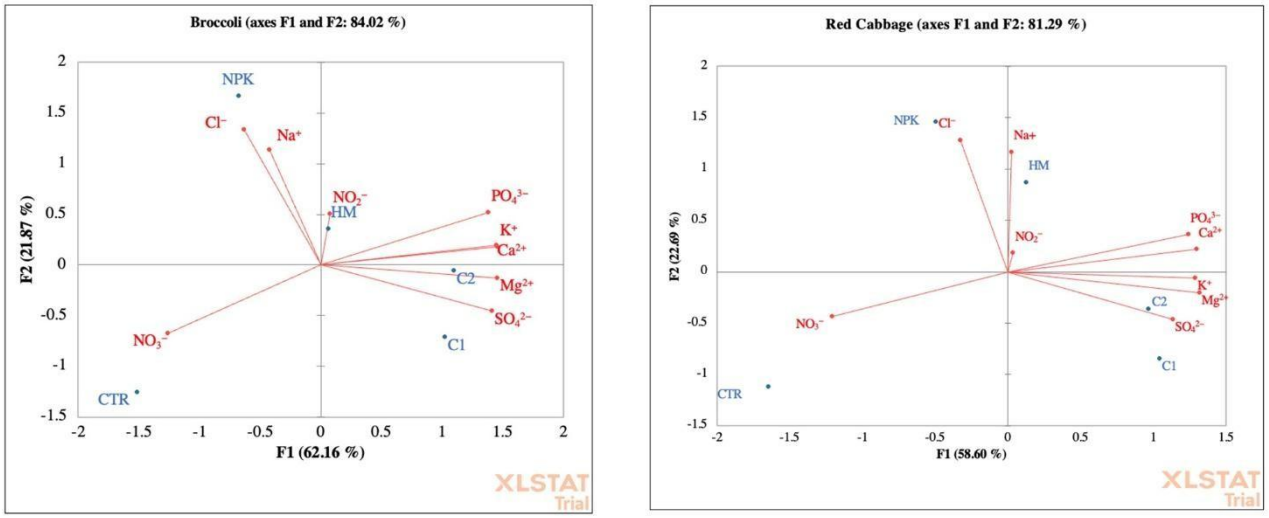
Table 6. Cations and anions concentrations (mg/L) detected 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw + 90% wet wastes Na^+ (sodium), K^+ (potassium), Ca^{2+} (calcium), Mg^{2+} (magnesium), Cl^- (chloride), NO_2^- (nitrite), NO_3^- (nitrate), PO_4^{3-} (phosphate), SO_4^{2-} (sulfate). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

Soil Cations	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2
Na^+	$0.124^{b} \pm 0.02$	$0.119^{b} \pm 0.08$	$0.101^{b} \pm 0.10$	$0.155^{b} \pm 0.09$	$0.91^{a} \pm 0.07$
K^+	$0.116^{c} \pm 0.07$	$0.165^{b} \pm 0.03$	$0.145^{bc} \pm 0.12$	$0.199^{a} \pm 0.11$	$0.290^{a} \pm 0.06$
Ca^{2+}	$0.254^{b} \pm 0.32$	$0.234^{b} \pm 0.22$	$0.346^{b} \pm 0.27$	$0.495^{b} \pm 0.19$	$3.53^{a} \pm 0.16$
Mg^{2+}	$0.019^{a} \pm 0.23$	$0.021^{a} \pm 0.31$	$0.027^{a} \pm 0.12$	$0.029^{a} \pm 0.22$	$0.027^{a} \pm 0.12$
Soil Anions	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2

Table 6. *Cont.*

Soil Cations	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2
Cl^-	0.222 ^{b} ± 0.23	0.206 ^{b} ± 0.34	0.208 ^{b} ± 0.21	0.310 ^{a} ± 0.07	0.298 ^{a} ± 0.02
NO_2^-	nd	nd	nd	nd	0.01 ± 0.01
NO_3^-	nd	nd	nd	nd	0.06 ± 0.02
PO_4^{3-}	nd	nd	nd	nd	0.003 ± 0.01
SO_4^{2-}	0.134 ^{c} ± 0.32	0.339 ^{b} ± 0.12	0.479 ^{b} ± 0.17	0.769 ^{b} ± 0.19	1.65 ^{a} ± 0.18

A PCA analysis demonstrated that C1 and C2 in red cabbage soil correlated with sulfate, magnesium, and potassium. NPK correlated with chloride; CTR with nitrate; and HM with the nitrite, phosphate, calcium, and sodium (Figure 4b). The scenario changed in soil with broccoli. C1 and C2 were both correlated with magnesium and sulfate. HM correlated as for red cabbage with the addition of potassium (Figure 4a).



(**a**) (**b**)

Figure 4. Principal component analyses of ions and cations soil with broccoli (**a**) and red cabbage (**b**). CTR (Control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes.

Organic carbon was the highest with composts. Total nitrogen was the greatest in NPK treatment. The C/N value was higher in soil fertilized with HM and composts with respect to CTR and NPK. WSP was the lowest in compost treatments, while DHA, MBC, bacteria, and actinomycetes were the highest. Fungi and FDA were more abundant in CTR and soil treated with NPK and HM (Table 7). A PCA analysis evidenced a strong positive correlation between C1 MBC, DHA, CEC, OC, and C/N, while C2 correlated better with bacteria, actinomycetes, and OC. HM and NPK were instead correlated with FDA, fungi, and WSP (Figure 5).

Pearson correlation coefficient evidenced a positive, significant similar tendency between organic matter, MBC, CEC, DHA, bacteria, and actinomycetes, suggesting that increasing the SOM amount also increased the amount of microbial biomass as well as the enzymes belonging to the oxo-reductase category, as also demonstrated by the increase in bacteria and actinomycete colonies (Table 8).

Table 7. Chemical and biochemical properties of soil located in Motta San Giovanni, 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes. WC (water content %), pH_{H_2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (dS/m); WSP = water-soluble phenols (µg TAE g^{-1} ds); OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (µg C g^{-1} f.s.); dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.s.), fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.s.), BACT (bacteria, UFC g^{-1} f.s.), FUN (fungi (UFC g^{-1} f.s.), ACT (Actinomycetes, UFC g^{-1} f.s.), CEC = cation exchange capacity ($cmol^{(+)}$ Kg^{-1}d.s.).

Soil Chemical Analyses	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2
WC (%)	$21.4^{b} \pm 0.02$	$22.2^{b} \pm 0.01$	$25.6^{a} \pm 0.03$	$25.2^{a} \pm 0.01$	$25.5^{a} \pm 0.01$
pH (H_2O)	$8.45^{a} \pm 0.12$	$8.46^{a} \pm 0.02$	$8.47^{a} \pm 0.05$	$8.44^{a} \pm 0.05$	$8.41^{a} \pm 0.01$
pH (KCl)	$7.1^{a} \pm 0.07$	$7.01^{a} \pm 0.06$	$6.99^{a} \pm 0.05$	$6.94^{a} \pm 0.04$	$6.97^{a} \pm 0.05$
EC	$350^{c} \pm 0.23$	$301^{c} \pm 0.22$	$297^{c} \pm 0.12$	$530^{b} \pm 0.17$	$740^{a} \pm 0.14$
OC	$1.78^{b} \pm 0.19$	$1.69^{b} \pm 0.22$	$2.13^{ab} \pm 0.11$	$2.9^{a} \pm 0.09$	$3.3^{a} \pm 0.09$
TN	$0.19^{a} \pm 0.17$	$0.23^{a} \pm 0.09$	$0.21^{a} \pm 0.13$	$0.19^{a} \pm 0.12$	$0.20^{a} \pm 0.11$
C/N	$9.4^{b} \pm 0.15$	$7.39^{c} \pm 0.15$	$19.1^{a} \pm 0.16$	$15.2^{a} \pm 0.11$	$16.5^{a} \pm 0.14$
WSP	$282^{b} \pm 0.32$	$320^{a} \pm 0.52$	$315^{a} \pm 0.42$	$138^{c} \pm 1.12$	$170^{c} \pm 0.92$
MBC	$433.3^{c} \pm 0.52$	$733^{b} \pm 0.17$	$798^{b} \pm 0.42$	$897.33^{a} \pm 0.52$	$961.4^{a} \pm 0.32$
FDA	$5.14^{a} \pm 0.44$	$5.44^{a} \pm 0.33$	$5.33^{a} \pm 0.27$	$4.88^{b} \pm 0.36$	$4.81^{b} \pm 0.18$
DHA	$20.1^{b} \pm 0.72$	$22.1^{b} \pm 0.32$	$24.1^{b} \pm 0.42$	$32.92^{a} \pm 0.32$	$38.09^{a} \pm 0.42$
BACT	$1.3 \times 10^{5\,c} \pm 1.42$	$1.1 \times 10^{5\,c} \pm 2.12$	$1.6 \times 10^{5\,c} \pm 3.32$	$5 \times 10^{5\,b} \pm 3.13$	$8.3 \times 10^{5\,a} \pm 2.12$
FUN	$4.6 \times 10^{4\,a} \pm 3.12$	$4.5 \times 10^{4\,a} \pm 1.42$	$4.6 \times 10^{4\,a} \pm 2.62$	$2.7 \times 10^{4\,b} \pm 2.11$	$3 \times 10^{4} \pm 2.02^{b}$
ACT	$5.7 \times 10^{4\,a} \pm 2.12$	$3.7 \times 10^{4\,b} \pm 4.12$	$6.7 \times 10^{4\,a} \pm 1.12$	$1.3 \times 10^{5\,c} \pm 3.16$	$1.5 \times 10^{5\,c} \pm 2.21$
CEC	$16^{b} \pm 0.13$	$12^{c} \pm 0.12$	$19^{ba} \pm 0.18$	$22^{a} \pm 0.11$	$22.9^{a} \pm 0.15$

Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 15) ± standard deviation.

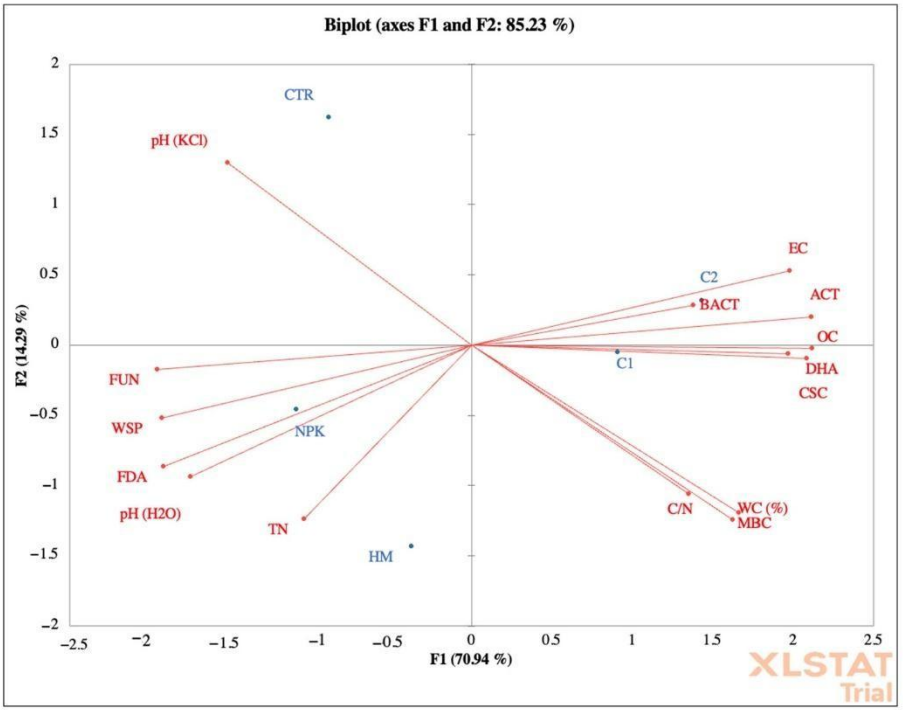


Figure 5. Principal component analyses of chemical and biochemical properties of soil located in Motta San Giovanni before fertilization. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes.

Table 8. Correlation matrix (Pearson) of chemical and biochemical properties of soil located in Motta San Giovanni, 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw and 90% wet wastes. Values in bold are different from 0 with a significance level of alpha = 0.05.

Variables	WC (%)	pH (H_2O)	pH (KCl)	EC	OC	TN	C/N	WSP	MBC	FDA	DHA	BACT	FUN	ACT	CEC
WC (%)	**1**	−0.315	−0.841	0.529	0.770	−0.175	**0.934**	−0.525	0.863	−0.473	0.740	0.403	−0.593	0.714	0.825
pH (H_2O)	−0.315	**1**	0.312	**−0.971**	−0.831	0.441	−0.189	0.794	−0.468	**0.879**	−0.847	−0.815	0.774	−0.848	−0.651
pH (KCl)	−0.841	0.312	**1**	−0.506	−0.715	−0.059	−0.602	0.602	**−0.959**	0.403	−0.752	−0.229	0.736	−0.655	−0.609
EC	0.529	**−0.971**	−0.506	**1**	**0.939**	−0.444	0.390	−0.860	0.641	**−0.912**	**0.947**	0.806	−0.861	**0.942**	0.787
OC	0.770	−0.831	−0.715	**0.939**	**1**	−0.463	0.637	**−0.895**	0.794	**−0.894**	**0.988**	0.677	**−0.913**	**0.992**	**0.921**
TN	−0.175	0.441	−0.059	−0.444	−0.463	**1**	−0.314	0.641	0.100	0.758	−0.341	−0.093	0.466	−0.565	−0.659
C/N	**0.934**	−0.189	−0.602	0.390	0.637	−0.314	**1**	−0.372	0.631	−0.406	0.561	0.351	−0.383	0.598	0.813
WSP	−0.525	0.794	0.602	−0.860	**−0.895**	0.641	−0.372	**1**	−0.591	**0.947**	−0.874	−0.394	**0.977**	**−0.934**	−0.819
MBC	0.863	−0.468	**−0.959**	0.641	0.794	0.100	0.631	−0.591	**1**	−0.456	0.841	0.485	−0.729	0.720	0.648
FDA	−0.473	**0.879**	0.403	**−0.912**	**−0.894**	0.758	−0.406	**0.947**	−0.456	**1**	−0.850	−0.558	**0.879**	**−0.942**	−0.861
DHA	0.740	−0.847	−0.752	**0.947**	**0.988**	−0.341	0.561	−0.874	0.841	−0.850	**1**	0.704	**−0.919**	**0.968**	0.851
BACT	0.403	−0.815	−0.229	0.806	0.677	−0.093	0.351	−0.394	0.485	−0.558	0.704	**1**	−0.416	0.636	0.521
FUN	−0.593	0.774	0.736	−0.861	**−0.913**	0.466	−0.383	**0.977**	−0.729	**0.879**	**−0.919**	−0.416	**1**	**−0.928**	−0.784
ACT	0.714	−0.848	−0.655	**0.942**	**0.992**	−0.565	0.598	**−0.934**	0.720	**−0.942**	**0.968**	0.636	**−0.928**	**1**	**0.932**
CEC	0.825	−0.651	−0.609	0.787	**0.921**	−0.659	0.813	−0.819	0.648	−0.861	0.851	0.521	−0.784	**0.932**	**1**

3.3. Crop Growth Data

In the presence of both composts, red cabbage exhibited a significant augmentation in leaf width, leaf area, leaf length, and plant height compared to the control, NPK, and HM treatments. The fruit-head diameter when C1 and C2 were applied shows an approximate 50% increase compared to the control and a 25% increase compared to HM and NPK. Productivity, measured in tons per hectare, experienced a noteworthy enhancement of 15% compared to NPK and HM in the presence of both composts. Notably, C1 demonstrated the most substantial effect on productivity, boasting a 35% increase compared to the control and a 30% increase compared to HM and NPK (Table 9).

Table 9. Growth parameters and productivity (tons per hectare) of red cabbage and broccoli grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes.

Red Cabbage	CTR	NPK	HM	C1	C2
Leaf humidity (%)	$84^{a} \pm 0.11$	$84^{a} \pm 0.62$	$86^{a} \pm 0.42$	$86^{a} \pm 0.32$	$85^{a} \pm 0.46$
Leaf width (cm)	$5.7^{a} \pm 0.56$	$8.8^{ab} \pm 0.25$	$1^{a} \pm 0.42$	$14^{a} \pm 0.15$	$13^{a} \pm 0.52$
Leaf length (cm)	$4.4^{a} \pm 0.12$	$7.8^{ab} \pm 0.41$	$10^{a} \pm 0.68$	$9.5^{a} \pm 0.42$	$10^{a} \pm 0.23$
Leaf area (cm^2)	$45^{c} \pm 0.25$	$65^{b} \pm 0.42$	$75^{b} \pm 0.13$	$96^{a} \pm 0.32$	$91^{a} \pm 0.15$
Plant height (cm)	$20^{c} \pm 0.125$	$30^{b} \pm 0.14$	$35^{b} \pm 0.12$	$43^{a} \pm 0.12$	$40^{a} \pm 0.43$
Head diameter (cm)	$10^{b} \pm 1.42$	$12^{a} \pm 2.32$	$12^{a} \pm 1.72$	$15^{a} \pm 2.52$	$15^{a} \pm 1.52$
Yield (Tons/ha)	$36^{b} \pm 1.51$	$42^{a} \pm 1.42$	$42^{a} \pm 1.32$	$49^{a} \pm 2.12$	$47^{a} \pm 2.32$

Table 9. *Cont.*

Red Cabbage	CTR	NPK	HM	C1	C2
Broccoli Calabrese	CTR	NPK	HM	C1	C2
Leaf humidity (%)	84 ^{a} ± 0.15	84 ^{a} ± 0.18	86 ^{a} ± 0.62	86 ^{a} ± 0.43	85 ^{a} ± 0.65
Leaf width (cm)	9 ^{a} ± 3.32	12 ^{a} ± 3.44	11 ^{a} ± 2.32	15 ^{a} ± 2.23	14 ^{a} ± 1.12
Leaf length (cm)	14 ^{a} ± 2.42	17 ^{a} ± 3.22	18 ^{a} ± 2.12	18 ^{a} ± 0.22	18 ^{a} ± 0.16
Leaf area (cm^2)	70 ^{b} ± 0.29	165 ^{a} ± 0.59	175 ^{a} ± 0.54	196 ^{a} ± 0.44	191 ^{a} ± 0.12
Plant height (cm)	50 ^{b} ± 0.34	60 ^{b} ± 0.14	65 ^{ab} ± 2.42	80 ^{a} ± 2.12	75 ^{a} ± 2.32
Head diameter (cm)	10 ^{b} ± 2.12	16 ^{a} ± 2.32	15 ^{a} ± 3.10	19 ^{a} ± 3.11	19 ^{a} ± 3.12
Yield (Tons/ha)	5 ^{c} ± 3.12	15 ^{b} ± 4.01	19 ^{ab} ± 2.12	22 ^{a} ± 4.2	21 ^{a} ± 3.11

Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 15) ± standard deviation.

Similarly, broccoli calabrese, when exposed to C1 and C2, exhibited a significant surge in growth. The leaf area tripled in comparison to the control, surpassing NPK and HM by 20%. Productivity, experiencing a fourfold increase compared to the control, surpassed NPK by 40% and HM by 15% (Table 9).

Chlorophyll (Table 10) data evidenced a greater amount of total chlorophyll and Cha/Chb ratio in broccoli and red cabbage treated with C1 and C2 with respect to CTR, HM, and NPK. Regarding the photosynthetic parameters of chlorophyll fluorescence, Fv, Fm, F0, and Y(NPQ) were the lowest in broccoli and red cabbage treated with both composts. Conversely, the Fv/Fm ratio, Y(NO), and ETR were instead the highest both in broccoli and red cabbage treated with both composts.

Table 10. Content of chlorophyll a (Chl a, mg 100 g^{-1}FW), chlorophyll b (Chl b, mg 100 g^{-1}FW), total chlorophyll (TChl, mg 100 g^{-1}FW), chlorophyll a/chlorophyll b (Chl a/Chl b) and photosynthetic parameters (FV, Fm, Y(NPQ), Y(NO) and ETR are expressed as μmol m^{-2} s^{-1}) in leaves of bed cabbage and broccoli calabrese.

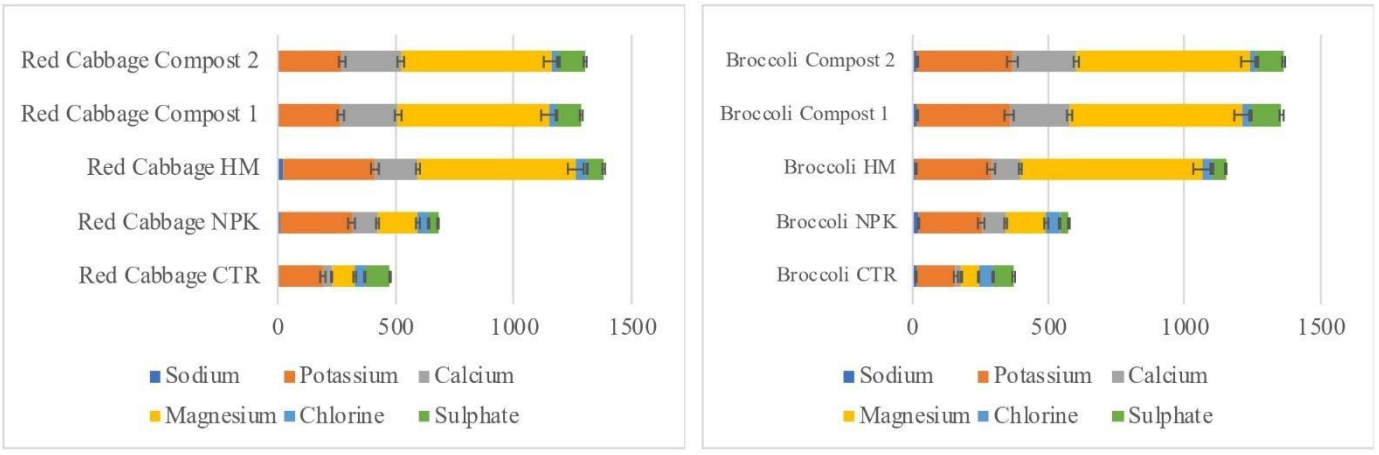
Broccoli Calabrese	CTR	NPK	HM	C1	C2
Chl a	114 ^{b} ± 0.43	120 ^{b} ± 0.12	142 ^{a} ± 0.02	158 ^{a} ± 0.12	167 ^{a} ± 0.19
Chl b	60 ^{a} ± 2.25	54 ^{a} ± 3.11	55 ^{a} ± 1.11	57 ^{a} ± 1.45	59 ^{a} ± 1.24
Chla/Chlb	1.9 ^{b} ± 1.11	2.2 ^{b} ± 1.12	2.58 ^{a} ± 1.54	2.77 ^{a} ± 1.12	2.83 ^{a} ± 1.02
T Chl	174 ^{b} ± 5.12	174 ^{b} ± 4.12	197 ^{ab} ± 3.14	215 ^{a} ± 4.11	226 ^{a} ± 2.12
FV	0.621 ^{b} ± 0.43	0.802 ^{ab} ± 0.22	1.004 ^{a} ± 0.12	1.007 ^{a} ± 0.52	1.107 ^{a} ± 0.23
Fm	0.939 ^{a} ± 0.65	1.077 ^{a} ± 0.21	1.423 ^{a} ± 0.22	1.222 ^{a} ± 0.61	1.343 ^{a} ± 0.36
F0	0.293 ^{b} ± 0.02	0.384 ^{ab} ± 0.02	0.528 ^{a} ± 0.11	0.534 ^{a} ± 0.12	0.544 ^{a} ± 0.74
Fv/Fm	0.661 ^{a} ± 0.02	0.74 ^{a} ± 0.01	0.71 ^{a} ± 0.12	0.82 ^{a} ± 0.01	0.82 ^{a} ± 0.01
Y(NPQ)	0.443 ^{a} ± 0.01	0.329 ^{a} ± 0.11	0.216 ^{a} ± 0.02	0.215 ^{a} ± 0.04	0.219 ^{a} ± 0.01
Y(NO)	0.235 ^{b} ± 0.02	0.215 ^{b} ± 0.01	0.344 ^{a} ± 0.01	0.397 ^{a} ± 0.03	0.361 ^{a} ± 0.04
ETR	21.21 ^{c} ± 0.12	28.84 ^{b} ± 0.16	35.24 ^{b} ± 0.14	41.26 ^{a} ± 0.13	39.54 ^{a} ± 0.14

Table 10. *Cont.*

Broccoli Calabrese	CTR	NPK	HM	C1	C2
Red Cabbage	CTR	NPK	HM	C1	C2
Chl a	94 ^{a} ± 0.56	100 a ± 1.52	112 ^{a} ± 4.12	118 ^{a} ± 4.67	117 ^{a} ± 5.12
Chl b	65 ^{a} ± 3.52	69 ^{a} ± 3.15	66 ^{a} ± 2.17	65 ^{a} ± 2.15	69 ^{a} ± 1.21
Chla/Chlb	1.45 ^{a} ± 0.01	1.45 ^{a} ± 0.42	1.47 ^{a} ± 0.13	1.81 ^{a} ± 0.13	1.71 ^{a} ± 0.11
T Chl	159 ^{b} ± 8.76	169 ^{a} ± 8.22	178 ^{a} ± 4.62	183 ^{a} ± 2.24	186 ^{a} ± 4.12
FV	0.644 ^{b} ± 0.02	0.776 ^{b} ± 0.01	1.016 ^{a} ± 0.01	1.027 ^{a} ± 0.02	1.144 ^{a} ± 0.02
Fm	0.899 ^{b} ± 0.03	1.000 ^{b} ± 0.25	1.227 ^{a} ± 0.26	1.392 ^{a} ± 0.11	1.465 ^{a} ± 0.12
F0	0.293 ^{b} ± 0.05	0.384 ^{b} ± 0.06	0.528 ^{a} ± 0.08	0.534 ^{a} ± 0.04	0.544 ^{a} ± 0.06
Fv/Fm	0.617 ^{a} ± 0.01	0.626 ^{a} ± 0.05	0.639 ^{a} ± 0.03	0.656 ^{a} ± 0.04	0.663 ^{a} ± 0.01
Y(NPQ)	0.433 ^{a} ± 0.02	0.409 ^{a} ± 0.08	0.216 ^{b} ± 0.03	0.225 ^{b} ± 0.07	0.256 ^{b} ± 0.09
Y(NO)	0.235 ^{b} ± 0.07	0.215 ^{b} ± 0.03	0.344 ^{a} ± 0.05	0.397 ^{a} ± 0.03	0.361 ^{a} ± 0.05
ETR	21.21 ^{c} ± 1.03	28.84 ^{b} ± 3.12	35.24 ^{b} ± 2.12	41.26 ^{a} ± 4.02	39.54 ^{a} ± 3.02

Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 15) ± standard deviation.

The ions were predominantly present in red cabbage and broccoli treated with both composts. Magnesium, calcium, and potassium were the most abundant cations in both crop species treated with composts C1 and C2 (Figure 6).



(**a**) (**b**)

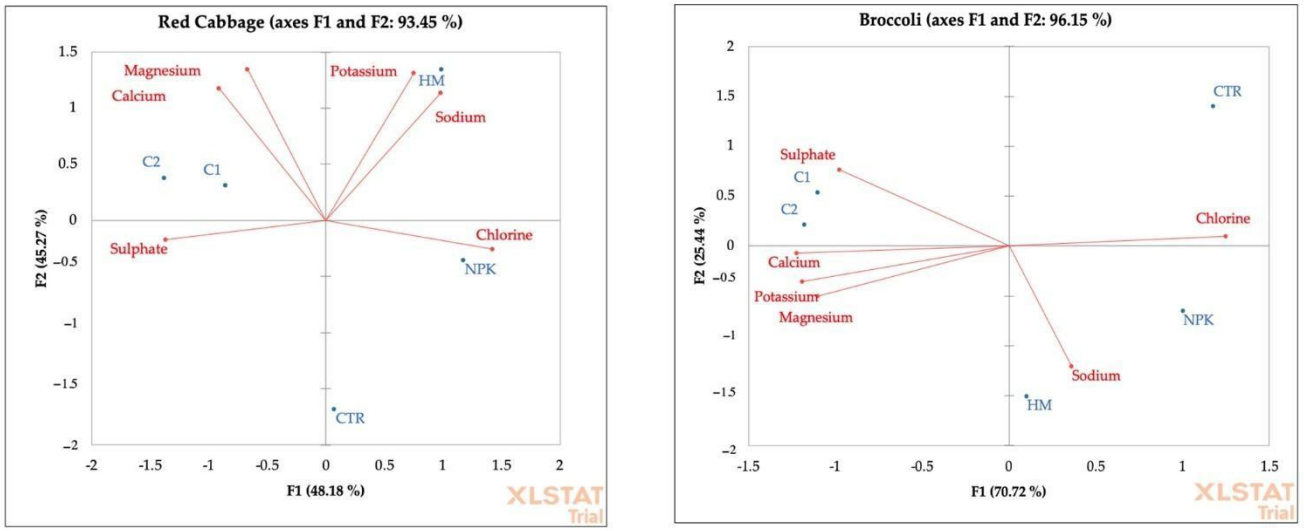
Figure 6. Bioaccumulation factor of red cabbage (**a**) and broccoli (**b**) grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes. Values are expressed as micrograms and are the mean of three replicates (n = 15) with errors standard.

Considering the bioaccumulation factor, red cabbage grown with composts 1 and 2 accumulated more magnesium, calcium, and sulfate in its leaves with respect to the other treatments. Similar results were observed for broccoli, with a greater increase even in potassium compared with the other treatments.

The best accumulation of ions has been observed in broccoli leaves treated with both composts (Figure 6).

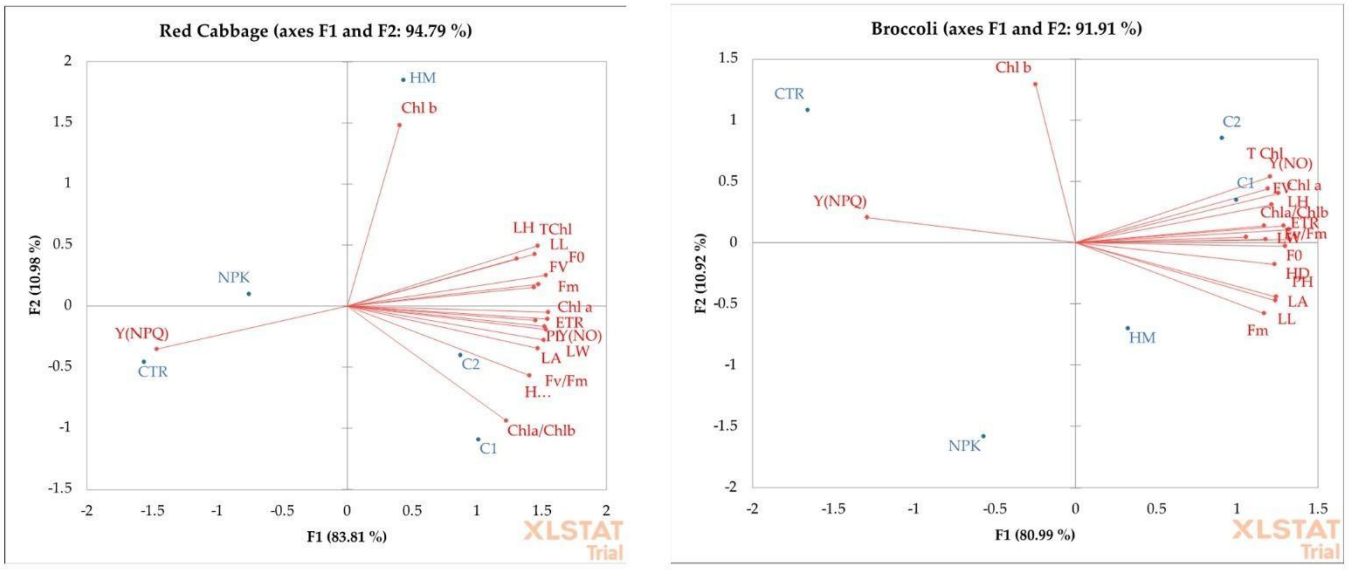
From the PCA, it emerged that broccoli cultivated with both composts accumulated sulfates, instead of HM, NPK, more sodium, and CTR chloride. (Figure 7b). The PCA related to red cabbage bioaccumulation factors evidenced an accumulation of magnesium and calcium with both composts; NPK and CTR showed an accumulation of Cl and HM of Na and K. (Figure 7a). Chlorophyll a and the photosynthesis parameters (ETR, Fm/Fv, and Y(NO) were mostly expressed in the presence of both composts in both crops. HM

correlated with total chlorophyll, chlorophyll B, F0, Fm, and Fv. No correlation between NPK and the parameters linked to photosynthesis activity has been found (Figure 8).



(**a**) (**b**)

Figure 7. PCA of ions and cations of red cabbage (**a**) and broccoli (**b**) grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes.



(**a**) (**b**)

Figure 8. Principal component analyses of the content of chlorophyll a (Chl a, mg 100 g^{-1}FW), chlorophyll b (Chl b, mg 100 g^{-1}FW), total chlorophyll (TChl, mg 100 g^{-1}FW), chlorophyll a/chlorophyll b (Chl a/Chl b) and photosynthetic parameters (FV, Fm, Y(NPQ), Y(NO), and ETR are expressed as µmol m^{-2} s^{-1}), in leaves of red cabbage (**a**) and broccoli (**b**) grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes.

4. Discussion

4.1. Compost Effects on Soil Chemical and Biological Properties

Composts 1 and 2 significantly affected soil properties, increasing EC and significantly enhancing organic matter content, CEC, microbial biomass, bacteria, and actinomycetes. The observed decrease in phenol content in compost-treated soils compared to the other treatments suggested that composts may have an impact on soil microorganisms and their metabolic activities. In fact, an increase in bacteria and actinomycetes has been observed as well. The observed significant increase in actinomycetes is of paramount importance due to their critical role in the cycling of organic matter. Actinomycetes serve as a natural barrier against a wide array of plant pathogens within the rhizosphere, effectively suppressing their growth. Moreover, they are adept at breaking down complex polymer mixtures present in deceased plants, animals, and fungi. This breakdown process facilitates the production of a diverse array of extracellular enzymes, which have been shown to significantly benefit crop production, enhancing both yield and health [36–38].

Further expanding on their beneficial impact, it has been demonstrated that actinomycetes not only augment the levels of nutrients and organic matter in the soil but also substantially increase the soil's microbial biomass [39]. This, in turn, boosts nitrogen availability, a critical component for plant growth, by stimulating the activity of essential nitrogen-metabolizing enzymes. The enhancement of nitrogen availability is particularly noteworthy, as it directly supports the growth and productivity of crops.

The multifaceted benefits of actinomycetes, from pathogen suppression and organic matter decomposition to nutrient enhancement and nitrogen availability, underscore their invaluable contribution to sustainable agriculture. By leveraging the positive roles of actinomycetes, it is possible to advance sustainable food-production practices that are both productive and environmentally friendly. This approach not only aims at achieving higher crop yields but also emphasizes biosafety and the preservation of ecological balance, making both composts a cornerstone in the pursuit of global food security and sustainable agricultural development.

The results are corroborated by the Pearson coefficient data, which reveal a positive correlation among microbial biomass carbon (MBC), organic content, water content (WC), dehydrogenase activity (DHA), and actinomycete populations. Such correlations were observed in soil samples collected from areas cultivating red cabbage and broccoli, where both types of compost were applied. These observations highlight the intricate interplay between soil characteristics and the microbial shifts following fertilizer application. The findings align with the research conducted by Arunrat et al. (2023) [40], which demonstrated that consistent fertilizer use and tillage methods over five years markedly enhanced the diversity and abundance of soil microbial communities.

The study reveals that both bacterial and actinomycete populations were significantly affected by Compost 2, as demonstrated by a PCA analysis (Figure 5). In contrast, Compost 1 was found to have a positive correlation with microbial biomass, water content, cation exchange capacity (CEC), and dehydrogenase activity (DHA). These findings indicate that are the characteristics of each type of compost that, influencing specific soil parameters, enhance or modify soil ecosystem functions.

This research suggests that it is not the inherent soil properties that remained consistent across different crops in this study, nor is it the type of crop cultivated that primarily influences soil ecosystem functioning. Instead, the key factor appears to be the raw material chosen for compost production. During the composting process, these raw materials are transformed into various bio-compounds, each possessing distinct specificities that can lead to different effects on the soil ecosystem.

In essence, the study highlights the critical role of compost composition in shaping soil health and functionality. By selecting appropriate compost materials, it is possible to tailor soil conditions to support desired ecosystem functions, thereby optimizing agricultural productivity and sustainability.

Notable changes in enzyme activities have been also observed. However, the correlation pattern between MBC and DHA with the addition of both composts evidenced the impact of composts on the soil's oxidative processes. It is important to note that both HM and NPK failed to exhibit a significant association with the chemical and biochemical attributes related to soil fertility.

In summary, these results offer crucial insights into the complex interplay among fertilizers, soil properties, and microbial interactions, which are fundamental for developing knowledgeable soil-management strategies and promoting sustainable agricultural practices. By closely monitoring these variables, we can evaluate soil health, microbial function, and nutrient cycling within variously treated soil ecosystems, thereby enhancing environmental stewardship. This approach not only aids in optimizing agricultural output but also in preserving ecological balance, ensuring a sustainable future for farming practices.

4.2. Compost Effects on Crop Yield and Quality

The changes noted had a beneficial impact on the yield and quality of red cabbage and broccoli. It was found that both yield and quality were linked to the levels of organic matter in the soil, a key factor in soil fertility and functionality. Organic matter contains trace elements vital for the needs of soil microorganisms, enhancing microbial activities. This, in turn, influences the interactions among soil microorganisms, which indirectly affects crop productivity. Such dynamics underscore the critical role of organic matter in supporting agricultural success, highlighting its importance in both soil health and crop performance.

The differences in both crops, grown with both composts, compared to the control and the other fertilizers were more evident in parameters related to leaf area, width, and length as well as head diameter. These results were supported by photosynthesis parameters and pigments that were increased in compost-treated crops than in the control and the NPK- and HM-treated crops. Total chlorophyll increased in crops grown with composts, probably because it correlated to the greatest leaf area. The method of chlorophyll fluorometry offers significant insights into the health of photosynthetic systems in plants by measuring the variable fluorescence of photosystem II [41]. Among the photosynthetic parameters, the ratio of variable fluorescence (Fv) to maximal fluorescence (Fm), known as Fv/Fm, serves as the most commonly utilized indicator. This ratio reflects the efficiency of primary light-energy conversion and the maximal efficiency of photosystem II (PSII) photochemistry [42,43]. The presence of negative effects on plants of external inputs is indicated by a reduced number of open reaction centers, leading to a decreased Fv/Fm ratio [44,45]. In this study, the lowest Fv/Fm values were observed in the control of both crops and in both crops grown with NPK and HM, indicating significant positive effects of composts on their photosynthetic efficiency. Y(II) serves as a metric for assessing plant efficiency, denoting the amount of energy utilized by photosystem II (PSII) under consistent photosynthetic lighting conditions, and is directly linked to the electron transport rate (ETR) and the plant's ability to assimilate carbon [46]. This relationship highlights the critical role of Y(II) in understanding the dynamics of photosynthesis, particularly in how efficiently a plant can convert light energy into chemical energy through PSII, further influencing its growth and productivity by affecting carbon assimilation processes. In the PCA (principal component analysis) of broccoli and red cabbage diagrams, the positioning of C1 and C2 in the right quadrants highlights the particular efficiency of composts on these cultivars. The spatial arrangement in the diagrams clearly illustrates how much weight they have on photosynthetic efficiency and, consequently, on crop growth and productivity. NPQ, which stands for non-photochemical quenching, acts as a measure of how plants dissipate excess light energy as heat within the antenna system to prevent photodamage. It is deemed a crucial short-term photoprotective mechanism in higher plants. With composts in both crops, NPQ values were observed to decrease across all cultivars, while increasing in the control and NPK-treated crops. This suggests that NPK may be the cause of oxidative damage to the photosynthetic apparatus of both crops. This interpretation is supported by the total chlorophyll content (TChl) data, which were the

lowest in the NPK- and HM-treated crops and in the controls of both crops. Crops treated with composts exhibited enhanced ion uptake, a finding substantiated by bioaccumulation-factor data, which indicated that these plants accumulated essential mineral nutrients critical for human health, including magnesium (Mg), calcium, potassium, and sulfate. Current food-supply statistics indicate that approximately half of the global population is at risk of dietary deficiencies in calcium (Ca) and Mg, with this figure escalating to over 95% in 16 African countries. The strategy of biofortifying crops with Mg and Ca has been recommended as a means to bolster dietary intakes for humans [47] as well as livestock [48,49], enhancing overall food-system nutrition. Despite their potential benefits, such biofortification practices have not yet been broadly implemented within agricultural production systems. In summary, both composts evidenced a positive effect on the crop quality of broccoli and red cabbage in comparison to horse manure and synthetic fertilizers.

5. Conclusions

In short, this study has successfully identified and evaluated environmentally friendly technologies for transforming organic wastes into fertilizers, aiming to enhance soil sustainability and crop yields. By comparing two compost formulations (Compost 1 and Compost 2) with horse manure (HM) and synthetic NPK fertilizers, and utilizing unfertilized soil as a control, the research provides compelling evidence on the efficacy of these organic amendments. Despite Compost 1 having a lower organic carbon content and enzyme activity compared to Compost 2, it emerged as the superior soil improver. It significantly increased the labile fraction of organic matter, the activity of oxidative enzymes, microbial biomass, and, importantly, crop yield. These findings underscore the potential of using specific compost formulations, particularly those with a high C/N ratio and effective humification of wet materials, as viable alternatives to conventional fertilizers. This approach not only promises to improve soil health and productivity but also contributes to the broader goal of sustainable agriculture by recycling organic wastes into valuable soil amendments.

Author Contributions: Conceptualization, A.M. (Adele Muscolo) and A.M. (Angela Maffia); methodology, F.M., M.O., C.M. and A.M. (Angela Maffia); software, A.M. (Angela Maffia) and S.B.; validation, A.M. (Adele Muscolo) and C.M.; formal analysis, F.M.; investigation, M.O.; data curation, C.M.; writing—original draft preparation, A.M. (Adele Muscolo); writing—review and editing, A.M. (Adele Muscolo) and A.M. (Angela Maffia); visualization, A.M. (Adele Muscolo); project administration, A.M. (Adele Muscolo); funding acquisition, A.M. (Adele Muscolo). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Calabrian Region, grant number DDL n° 16315 del 13-12-2022, POR CALABRIA FESR-FSE 2014–2020 ASSE I—PROMOZIONE DELLA RICERCA E DELL'INNOVAZIONE.

Data Availability Statement: Suggested data availability statements are available at "https://iris.unirc.it/" (accessed on 8 April 2024).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Muhie, S.H. Novel approaches and practices to sustainable agriculture. *J. Agric. Food Res.* **2022**, *10*, 100446. [CrossRef]
2. Alexandratos, N.; Bruinsma, J. *World Agriculture towards 2030/2050: The 2012 Revision*; ESA Working Papers 12-03; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2012.
3. Willett, W.C.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S.J.; Garnett, T.; Tilman, D.; DeClerck, F.; Wood, A.; et al. Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* **2019**, *393*, 447–492. [CrossRef]
4. Hassan, N.; Wahed, N.H.A.E.; Abdelhamid, A.O.; Ashraf, M.; Abdelfattah, E.A. Composting: An eco-friendly solution for organic waste management to mitigate the effects of climate change. *Innovare J. Soc. Sci.* **2023**, *11*, 1–7. [CrossRef]
5. EU. Commission Implementing Regulation (EU) 2021/1165 Authorising Certain Products and Substances for Use in Organic Production and Establishing Their Lists. 2021. Available online: https://eur-lex.europa.eu/eli/reg_impl/2021/1165/oj (accessed on 15 July 2021).

6. Muscolo, A.; Papalia, T.; Settineri, G.; Mallamaci, C.; Jeske-Kaczanowska, A. Are raw materials or composting conditions and time that most influence the maturity and/or quality of composts? Comparison of obtained composts on soil properties. *J. Clean. Prod.* **2018**, *195*, 93–101. [CrossRef]
7. Goldan, E.; Nedeff, V.; Bârsan, N.; Culea, M.; Panainte-Lehăduș, M.; Mosneguțu, E.; Tomozei, C.; Chițimuș, D.; Irimia, O. Assessment of Manure Compost Used as Soil Amendment—A review. *Processes* **2023**, *11*, 1167. [CrossRef]
8. Ghimire, S.; Chhetri, B.P.; Shrestha, J. Efficacy of different organic and inorganic nutrient sources on the growth and yield of bitter gourd (*Momordica charantia* L.). *Heliyon* **2023**, *9*, e22135. [CrossRef] [PubMed]
9. Bonanomi, G.; De Filippis, F.; Zotti, M.; Idbella, M.; Cesarano, G.; Al-Rowaily, S.L.; Abd-ElGawad, A.M. Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield. *Appl. Soil Ecol.* **2020**, *156*, 103714. [CrossRef]
10. Suman, J.; Rakshit, A.; Ogireddy, S.D.; Singh, S.; Gupta, C.; Chandrakala, J. Microbiome as a key player in sustainable agriculture and human health. *Front. Soil Sci.* **2022**, *2*, 821589. [CrossRef]
11. Insam, H.; Merschak, P. Nitrogen leaching from forest soil cores after amending organic recycling products and fertilizers. *Waste Manag. Res.* **1997**, *15*, 277–291. [CrossRef]
12. Mallakpour, S.; Sirous, F.; Hussain, C.M. Sawdust, a versatile, inexpensive, readily available bio-waste: From mother earth to valuable materials for sustainable remediation technologies. *Adv. Colloid Interface Sci.* **2021**, *295*, 102492. [CrossRef]
13. Ayilara, M.S.; Olanrewaju, O.S.; Babalola, O.O.; Odeyemi, O. Waste Management through Composting: Challenges and Potentials. *Sustainability* **2020**, *12*, 4456. [CrossRef]
14. Nemet, F.; Perić, K.; Lončarić, Z. Microbiological activities in the composting process: A review. *Columella* **2021**, *8*, 41–53. [CrossRef]
15. Liang, C.; Das, K.C.; McClendon, R.W. The Influence of Temperature and Moisture Contents Regimes on the Aerobic Microbial Activity of a Biosolids Composting Blend. *Bioresour. Technol.* **2003**, *86*, 131–137. [CrossRef] [PubMed]
16. ANPA-National Agency for Environmental Protection Guidelines. *"Methods of Compost Analysis", Manuals and Guidelines 3/2001*; 6334 manuali 3; SPED S.r.l.: Roma, Italy, 2001; ISBN 88-448-0258-9.
17. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951. [CrossRef]
18. Perucci, P. Enzyme activity and microbial biomass in a field soil amended with municipal 460 refuse. *Biol. Fertil. Soils* **1992**, *14*, 54–60. [CrossRef]
19. Von Mersi, W.; Schinner, F. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
20. Box, J.D. Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
21. Hendershot, W.H.; Duquette, M. A Simple Barium Chloride Method for Determining Cation Exchange Capacity and Exchangeable Cations. *Soil Sci. Soc. Am. J.* **1986**, *50*, 605–608. [CrossRef]
22. Gariglio, N.; Buyatti, M.; Pilatti, R.A.; Russia, D.E.G.; Acosta, M.R. Use of a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *N. Z. J. Crop Hortic. Sci.* **2002**, *30*, 135–139. [CrossRef]
23. Zucconi, F.; Pera, A.; Forte, M.; De Bertoldi, M. Evaluating toxicity of immature compost. *BioCycle* **1981**, *22*, 54–57.
24. IUSS Working Group WRB. World Reference Base for Soil Resources. In *International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*, 4th ed.; International Union of Soil Sciences (IUSS): Vienna, Austria, 2022.
25. Bouyoucos, G.J. Hydrometer method improved for making particle size analysis of soils. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
26. Muscolo, A.; Settineri, G.; Papalia, T.; Attinà, E.; Basile, C.; Panuccio, M.R. Anaerobic co-digestion of recalcitrant agricultural wastes: Characterizing of biochemical parameters of digestate and its impacts on soil ecosystem. *Sci. Total Environ.* **2017**, *586*, 746–752. [CrossRef]
27. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
28. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoff in organishen Kopern. *Anal. Chem.* **1883**, *22*, 354–358.
29. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
30. Insam, H.; Goberna, M. *Section 4 Update: Use of Biolog® for the Community Level Physiological Profiling (CLPP) of Environmental Samples*; Springer eBooks: Berlin/Heidelberg, Germany, 2008; pp. 2755–2762. [CrossRef]
31. Waksman, S.A. *Soil Microbiology*; John Wiley & Sons: New York, NY, USA, 1952.
32. Johnson, L.F.; Curl, E.A. *Methods for the Research on Ecology of Soil-Borne Plant Pathogens*; Burgess Publishing Co.: Minneapolis, MN, USA, 1972.
33. Hiscox, J.; Israelstam, G.F. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **1979**, *57*, 1332–1334. [CrossRef]
34. Anonymous. *Recommended Practice for Chemical Analysis by Ion Chromatography*; Australian Standard AS 3741: Sidney, Australia, 1990.
35. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef] [PubMed]

36. Bhatti, A.A.; Haq, S.; Bhat, R.A. Actinomycetes benefaction role in soil and plant health. *Microb. Pathog.* **2017**, *111*, 458–467. [CrossRef] [PubMed]
37. Charousová, I.; Javoreková, S.; Medo, J.; Schade, R. Characteristic of selected soil streptomycetes with antimicrobial potential against phytopathogenic microorganisms. *J. Microbiol. Biotechnol. Food Sci.* **2016**, *5*, 64–68. [CrossRef]
38. Charousová, I.; Medo, J.; Halenárová, E.; Javoreková, S. Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands. *J. Adv. Pharm. Technol. Res.* **2017**, *8*, 46–51. [CrossRef]
39. AbdElgawad, H.; Abuelsoud, W.; Madany, M.M.Y.; Selim, S.; Zinta, G.; Mousa, A.S.; Hozzein, W.N. Actinomycetes Enrich Soil Rhizosphere and Improve Seed Quality as well as Productivity of Legumes by Boosting Nitrogen Availability and Metabolism. *Biomolecules* **2020**, *10*, 1675. [CrossRef] [PubMed]
40. Arunrat, N.; Sansupa, C.; Sereenonchai, S.; Hatano, R. Stability of soil bacteria in undisturbed soil and continuous maize cultivation in Northern Thailand. *Front. Microbiol.* **2023**, *14*, 1285445. [CrossRef] [PubMed]
41. Ivanov, D.A.; Bernards, M.A. Chlorophyll fluorescence imaging as a tool to monitor the progress of a root pathogen in a perennial plant. *Planta* **2015**, *243*, 263–279. [CrossRef] [PubMed]
42. Adhikari, K.; Owens, P.R.; Libohova, Z.; Miller, D.M.; Wills, S.; Nemecek, J.L. Assessing soil organic carbon stock of Wisconsin, USA and its fate under future land use and climate change. *Sci. Total Environ.* **2019**, *667*, 833–845. [CrossRef]
43. Hussain, M.I.; Reigosa, M.J. A chlorophyll fluorescence analysis of photosynthetic efficiency, quantum yield and photon energy dissipation in PSII antennae of *Lactuca sativa* L. leaves exposed to cinnamic acid. *Plant Physiol. Biochem.* **2011**, *49*, 1290–1298. [CrossRef] [PubMed]
44. Moustakas, M.; Bayçu, G.; Gevrek, N.; Moustaka, J.; Csatári, I.; Rognes, S.E. Spatiotemporal heterogeneity of photosystem II function during acclimation to zinc exposure and mineral nutrition changes in the hyperaccumulator *Noccaea caerulescens*. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 6613–6624. [CrossRef]
45. Oxborough, K.; Baker, N.R. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components–calculation of qP and Fv0/Fm0 without measuring Fo0. *Photosynth. Res.* **1997**, *54*, 135–142. [CrossRef]
46. Del Pozo, A.; Pérez, P.P.; Gutiérrez, D.G.; Alonso, A.; Morcuende, R.; Martínez-Carrasco, R. Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. *Environ. Exp. Bot.* **2007**, *59*, 371–380. [CrossRef]
47. White, P.J.; Broadley, M.R. Biofortification of crops with seven mineral elements often lacking in human diets—Iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol.* **2009**, *182*, 49–84. [CrossRef]
48. Kumssa, D.B.; Lovatt, J.A.; Graham, N.; Palmer, S.; Hayden, R.; Wilson, L.; Young, S.D.; Lark, R.M.; Penrose, B.; Ander, E.L.; et al. Magnesium biofortification of Italian ryegrass (*Lolium multiflorum* L.) via agronomy and breeding as a potential way to reduce grass tetany in grazing ruminants. *Plant Soil* **2019**, *457*, 25–41. [CrossRef]
49. Penrose, B.; Lovatt, J.A.; Palmer, S.; Thomson, R.; Broadley, M.R. Revisiting variation in leaf magnesium concentrations in forage grasses for improved animal health. *Plant Soil* **2020**, *457*, 43–55. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Chapter 4 - Circular Fertilization Strategy Using Sulphur with Orange Waste Enhances Soil Health and Broccoli Nutritional and Nutraceutical Quality in Mediterranean Systems

Mariateresa Oliva [1], Federica Marra[1], Ludovica Santoro[1], Santo Battaglia[1], Carmelo Mallamaci[1] and Adele Muscolo[1*]

[1] Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy.

*Correspondence: amuscolo@unirc.it

Applied Sciences

https://doi.org/10.3390/app15169010

Received: 18 July 2025

Revised: 13 August 2025

Accepted: 14 August 2025

Published: 15 August 202

applied sciences

MDPI

Article

Circular Fertilization Strategy Using Sulphur with Orange Waste Enhances Soil Health and Broccoli Nutritional and Nutraceutical Quality in Mediterranean Systems

Mariateresa Oliva, Federica Marra, Ludovica Santoro, Santo Battaglia, Carmelo Mallamaci and Adele Muscolo *

Department of AGRARIA, "Mediterranea" University, 89122 Reggio Calabria, Italy; mariateresa.oliva@unirc.it (M.O.); federica.marra@unirc.it (F.M.); ludovica.santoro@unirc.it (L.S.); santo.battaglia@unirc.it (S.B.); carmelo.mallamaci@unirc.it (C.M.)
* Correspondence: amuscolo@unirc.it

Abstract

Fertilization strategies are pivotal in sustainable agriculture, affecting both soil health and crop quality. This study investigated the impact of a circular fertilization approach based on agro-industrial residues—specifically, a blend of sulfur bentonite and orange processing waste (RecOrgFert PLUS)—on soil physicochemical and biological properties, as well as the nutritional and nutraceutical quality of broccoli (*Brassica oleracea* var. *italica*) grown in Mediterranean conditions (Condofuri, Southern Italy). The effects of RecOrgFert PLUS were compared with those of a synthetic NPK fertilizer, an organic fertilizer (horse manure), and an unfertilized control. Results demonstrated that RecOrgFert PLUS significantly improved soil organic carbon (3.37%), microbial biomass carbon (791 μg C g^{-1}), and key enzymatic activities, indicating enhanced soil biological functioning. Broccoli cultivated under RecOrgFert PLUS also exhibited the highest concentrations of health-promoting compounds, including total phenols (48.87 mg GAE g^{-1}), vitamin C (51.93 mg ASA 100 g^{-1}), and total proteins (82.45 mg BSA g^{-1}). This work provides novel evidence that combining elemental sulphur with orange processing waste not only restores soil fertility but also boosts the nutraceutical and nutritional value of food crops. Unlike previous studies focusing on soil or plant yield alone, this study uniquely integrates soil health indicators with bioactive compound accumulation in broccoli, highlighting the potential of circular bio-based fertilization in functional food production and Mediterranean agroecosystem sustainability.

Keywords: agro-environmental sustainability; sustainable fertilizers; broccoli quality; soil quality

check for updates

Academic Editors: Marta Mesías and Tiane Finimundy

Received: 18 July 2025
Revised: 13 August 2025
Accepted: 14 August 2025
Published: 15 August 2025

Citation: Oliva, M.; Marra, F.; Santoro, L.; Battaglia, S.; Mallamaci, C.; Muscolo, A. Circular Fertilization Strategy Using Sulphur with Orange Waste Enhances Soil Health and Broccoli Nutritional and Nutraceutical Quality in Mediterranean Systems. *Appl. Sci.* **2025**, *15*, 9010. https://doi.org/10.3390/app15169010

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Soil fertility is a cornerstone of sustainable agriculture, directly influencing plant productivity, nutrient availability, and the diversity and function of soil microbial communities [1,2]. In recent decades, the global push to increase food production has driven the intensification of agricultural practices, often through a heavy reliance on synthetic fertilizers. While these inputs have contributed to increased yields, their long-term use has led to negative consequences including soil degradation, nutrient imbalances, suppression of beneficial microorganisms, and environmental issues such as nitrate leaching and

greenhouse gas emissions [3,4]. To address these challenges, sustainable soil fertility management practices are urgently needed. A promising approach involves the use of organic fertilizers—such as compost, and animal manures—which not only supply nutrients but also improve soil structure, increase organic matter, and stimulate microbial activity [2,5,6].

Organic fertilizers typically release nutrients more slowly than synthetic counterparts, enhancing nutrient use efficiency while reducing the risk of leaching and volatilization losses [7]. Among these organic amendments, those derived from agricultural wastes or biochar are gaining recognition for their dual role in improving soil fertility and recycling organic wastes [5,8]. These inputs have been shown to enhance soil water retention, aeration, cation exchange capacity, and microbial biomass, which are particularly beneficial in Mediterranean agroecosystems where climatic and edaphic constraints often limit productivity [2,5]. Numerous studies have demonstrated that long-term balanced fertilization—particularly when incorporating sulphur (S) alongside primary macronutrients such as nitrogen (N), phosphorus (P), and potassium (K)—can significantly reduce nitrous oxide (N_2O) emissions without compromising crop productivity, thereby contributing to more sustainable agricultural systems [4,9,10]. These findings highlighted the critical role of elemental S, not only in mitigating greenhouse gas emissions but also in maintaining and enhancing soil fertility.

S is increasingly recognized as an essential nutrient for optimal soil function and plant health. It plays a key role in enzymatic reactions, protein synthesis, and the mobilization of vital nutrients such as N, P, and K, thereby improving nutrient uptake efficiency and overall soil–plant nutrient dynamics [3,7]. Despite its importance, S has often been overlooked in fertilization regimes, largely due to the historic reliance on atmospheric deposition from industrial pollution to meet crop requirements. However, modern agricultural practices—including intensified cultivation, reduced use of organic amendments, widespread adoption of S-free synthetic fertilizers, and significant declines in atmospheric S deposition due to cleaner air policies—have collectively led to widespread S deficiency in many arable soils [8,11]. This deficiency is increasingly associated with reduced crop yields, poor nutrient balance, and diminished soil microbial activity. Consequently, there is a growing consensus that S should be re-evaluated and integrated more systematically into sustainable nutrient management strategies, particularly in regions like the Mediterranean where soil degradation and nutrient imbalances are common. The incorporation of elemental S or S-enriched organic amendments can not only restore S levels but also stimulate microbial communities responsible for S oxidation and nutrient mineralization, enhancing both soil health and plant resilience [4,5]. Thus, S fertilization should be considered a critical component of holistic soil fertility management aimed at improving agroecosystem sustainability, nutrient use efficiency, and environmental stewardship.

Given the multifaceted benefits that can come from the integration of organic components with S in a sustainable fertilization strategy that is able to increase the health properties of broccoli, this study aims to compare the effects of synthetic fertilizers (NPK), horse manure (HM), and a mineral–organic fertilizer (RecOrgFert PLUS)—composed of elemental S, hydrocarbon residues from refining processes, and orange waste residues from the food industry—on key soil parameters, microbial activity, and the nutritional and nutraceutical quality of broccoli. Previous research by Muscolo et al. [8] using S bentonite plus orange waste primarily emphasized the improvements in soil fertility and nutrient cycling and enhanced plant yield and quality in other crops.

In addition to improving soil health, fertilization strategies also influence the nutritional and nutraceutical quality of crops.

Broccoli (*Brassica oleracea* var. *italica*), a widely consumed vegetable in Mediterranean regions, is highly valued for its content of antioxidants, vitamins, and glucosinolates, and

is particularly sensitive to soil nutrient dynamics [12,13]. Several studies have shown that organic fertilization can increase the accumulation of health-promoting phytochemicals such as phenolic compounds and vitamin C in broccoli, thereby improving its nutritional and nutraceutical quality [13–15]. Moreover, the influence of S fertilization on the accumulation of glucosinolates and other S-containing compounds in *Brassica* species (e.g., broccoli, cabbage, kale, mustard, rapeseed) is well-documented. S is not just a nutrient—it is a precursor and regulator in the biosynthesis of key secondary metabolites containing S, which are associated with plant defense and human health benefits (e.g., anticancer effects). This is particularly relevant in Mediterranean systems, where climate variability and limited water resources demand resilient cropping systems supported by robust soil microbial networks.

This is the first study to evaluate the efficiency of this innovative sulfur–bentonite-based fertilizer at the field scale, linking soil biochemical properties related to soil health with the nutraceutical compounds that determine broccoli quality. Sulfur bentonite represents a promising strategy for sustainable fertilization because it combines elemental sulfur with bentonite clay, ensuring a gradual oxidation of sulfur by soil microorganisms and a controlled release of sulfate. This slow-release mechanism not only improves sulfur availability throughout the crop cycle but also stimulates microbial activity, enhances nutrient cycling, and contributes to the resilience of soil ecosystems. Furthermore, the incorporation of organic residues, such as orange waste, promotes circular economy principles by valorizing agro-industrial byproducts and reducing dependency on synthetic inputs. Beyond improving soil health, sulfur plays a crucial role in the biosynthesis of sulfur-containing secondary metabolites, such as glucosinolates, which are key nutraceutical compounds in broccoli. By integrating soil health indicators with nutritional quality parameters, this study aims to clarify whether waste-based fertilizers can deliver dual benefits: maintaining agroecosystem functionality and enhancing the nutritional and nutraceutical quality of food. The findings provide valuable evidence for the development of fertilization strategies that support both sustainability and human health.

2. Materials and Methods

2.1. Soil Treatment

The experimental trial was carried out in Falcone Farm Condofuri, Reggio Calabria, located at 339 m above sea level. The soil at the site was sandy loam with a texture composition of 88% sand, 9% silt, and 3% clay, according to the FAO system criteria [16].

The experimental area covered a total of two hectares, subdivided into plots of 20 square meters each. Different fertilization treatments were applied across the plots, with each treatment replicated six times to ensure the reliability and statistical robustness of the results. The fertilization treatments included an unfertilized control soil (CTR), serving as the baseline for comparison; an organic treatment based on horse manure (HM), applied at a rate of 4300 kg ha^{-1}; a synthetic fertilizer with a nitrogen–phosphorous–potassium (NPK) formulation of 20:10:10, distributed at 1700 kg ha^{-1}; and waste-based fertilizer (RecOrgFert PLUS) produced by recycling elemental S, hydrocarbon refining process residues, and orange residues from the food industry. The RecOrgFert PLUS fertilizer was produced by SBS Steel Belt Systems srl in tablet form (3–4 mm), as described by Muscolo [17]. Elemental sulfur (S) was the main component of the mixture, which also included bentonite (B) and orange residues (O) derived from the food industry. The formulation underwent analytical testing to ensure the absence of pathogens (total coliforms, fecal coliforms, *Salmonella* spp., and *Escherichia coli*) and heavy metals, in order to avoid any polluting impact on the soil [18]. The results confirmed the absence of both pathogens and heavy metals [18]. This latter formulation was applied at a dose of 476 kg ha^{-1}.

These treatments were selected to compare traditional, synthetic, and novel sustainable fertilization strategies on Calabrian sprouting broccoli.

2.2. Chemical and Biochemical Soil Analysis

Texture was determined using the hydrometer method of Bouyoucos [19]. Soil moisture content was quantified by oven-drying the samples at 105 °C, following the standard method proposed by AOAC [20]. Electrical conductivity (EC) was assessed using a Hanna Instruments conductivity meter on a soil-to-water suspension prepared at a 1:5 ratio and mechanically stirred at 15 rpm for one hour. Soil pH was measured in distilled water using a 1:2.5 soil-to-solution ratio and a glass electrode. Organic carbon and total nitrogen were determined using a LECO CN628 (St. Joseph, MI, USA) elemental analyzer, and the resulting carbon values were converted into organic matter by multiplying for an empiric value of 1. The carbon-to-nitrogen (C/N) ratio was then calculated. In addition, cation exchange capacity (CEC) was determined according to the method of Mehlich et al. [21].

Water-soluble total phenols, including both monomeric and polymeric forms, were determined using the Folin–Ciocalteu reagent [22], with gallic acid used as a standard. Results were expressed in terms of gallic acid equivalents (µg GAE per gram of dry weight). Dehydrogenase activity (DHA) was measured using iodonitrotetrazolium chloride, according to the method of von Mersi and Schinner [23]. Catalase activity was assessed volumetrically by quantifying the oxygen released after incubating the soil with hydrogen peroxide for three minutes, as described by Beck [24]. Fluorescein diacetate hydrolase (FDA) activity, which provides a general indication of enzymatic potential in the soil, was analyzed following the procedure reported by Dick et al. [25]. Microbial biomass carbon (MBC) was quantified via the chloroform fumigation–extraction method outlined by Vance et al. [26], using field-moist samples equivalent to 20 g dry weight. Both fumigated and unfumigated soil extracts were analyzed for soluble organic carbon using the Walkley–Black method [27]. MBC was estimated by calculating the difference between the organic carbon extracted from fumigated and unfumigated soils, applying an extraction efficiency coefficient of 0.38 to convert soluble carbon into biomass carbon [26]. Cations and anions were identified using ion chromatography (Thermo Scientific Dionex ICS-1100, Sunnyvale, CA, USA). For anion analysis, 0.5 g of dried sample was mixed with 50 mL of an anionic extracting solution (3.5 mM Na_2CO_3/$NaHCO_3$) and stirred for 20 min. The resulting extract was then filtered before undergoing chromatographic analysis. To determine cation content, 1 g of dried sample was incinerated at 550 °C for 5–6 h in a porcelain crucible. The resulting ash was digested in 1 M HCl at 100 °C for 30 min. This solution was filtered and analyzed via ion chromatography using a 20 mM methanesulfonic acid eluent.

2.3. QBS–ar Index

Soil samples were collected during the flowering and post-harvest phases. The sampling depth ranged from 0 to 35 cm. For each sampling, soil was collected from multiple points within each plot to obtain three replicates per treatment. One kilogram of fresh soil from each replicate was used for the extraction of soil microarthropods using a Tüllgren extractor, following the method of Bano and Roy ì [28]. The soil was placed in a sieve with a height of sixty millimeters and a diameter of two hundred and twenty millimeters, featuring a two-millimeter mesh, positioned above a funnel that directed the microarthropods into a container filled with an alcohol and glycerin solution in a 2:1 ratio. The extractor was placed under a heat source, a 40- or 60-watt light bulb located twenty centimeters away and kept in operation for ten days to facilitate the migration of microarthropods into the container. Collected specimens were observed under a stereomicroscope (20×–40× magnification). Each individual was classified according to its Biological Form and assigned an

Eco-Morphological Index (EMI) score, as described by Angelini et al. [29]. In cases where multiple forms from the same group were present, only the individual with the highest EMI score was recorded.

The QBS-ar index (Biological Quality of Soil based on Arthropods) was calculated as the sum of EMI scores of all taxa found in each sample. A higher QBS-ar value indicates a more biodiverse and functionally healthy soil environment.

2.4. Broccoli Nutritional and Nutraceutical Analysis

The total phenol content was determined using the Folin–Ciocalteu reagent [22], following the procedure described by Velioglu et al. [30]. Total flavonoid content was measured using a colorimetric method based on aluminum chloride, according to the protocol developed by Djeridane et al. [31]. To assess antioxidant activity, two complementary methods were employed. The ABTS+ radical cation decolorization assay was conducted following the method of Re et al. [32], while the DPPH method, which relies on the stable free radical 2,2-diphenyl-1-picrylhydrazyl, was performed according to Barreca et al. [33]. Total antioxidant capacity (TAC) was quantified based on the method described by Prieto et al. [34]. Vitamin E content was detected following the Prieto et al. [34] method. The concentration of vitamin C (ascorbic acid) was determined using the colorimetric method as described by Davies and Masten [35]. Total protein was detected using the Bradford method, following the procedure described by Kruger [36]. Total carbohydrates were measured using the phenol-Sic acid method according to Hedge et al. [37].

2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed for all datasets. One-way ANOVA followed by Tukey's honestly significant difference (HSD) test was used to evaluate the effects of fertilizers on the various measured parameters. Pairwise comparisons were conducted to assess the effects of fertilizers on each individual parameter. To explore the relationships between different fertilizers and broccoli parameters, the datasets were analyzed using principal component analysis (PCA). In addition, Pearson's correlation analysis was carried out to assess the strength and direction of linear associations among the considered parameters. Statistical analyses were performed using MATLAB (version R2024b, The MathWorks Inc., Natick, MA, USA). Effects were considered significant at $p \leq 0.05$.

3. Results

3.1. Soil Physicochemical Properties

Table 1 shows the soil characteristics prior to the application of fertilization, reported in in the first column as time 0 (T0). The low values of water content (8.25%), organic carbon (1.13%), cation exchange capacity (15.0 cmol (+) kg^{-1} ds), and microbial biomass carbon (205 μg C g^{-1}) reflect a condition of limited soil fertility. Fertilization significantly affected the physico-chemical and biochemical properties of the soil, with variable impacts depending on the type of amendment applied.

Soil water content (WC) increased significantly in the presence of RecOrgFert PLUS (14.5%) and NPK (14.2%) compared to the control (10.1%). Organic carbon (OC) content also increased after fertilization, with RecOrgFert PLUS showing the highest value (3.37%), followed by horse manure (2.54%) and NPK (2.3%). All treatments resulted in significantly higher OC levels than the control (1.17%).

Table 1. Chemical and biochemical properties of soil located in Condofuri: T0 = time zero; CTR (control) soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. WC = water content (%); pH= potential of hydrogen; EC = electrical conductivity (dS/m); OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon–nitrogen ratio; OM = organic matter (%); WSP = water-soluble phenols (μg TAE g^{-1} ds); CEC = cation exchange capacity (cmol (+) kg^{-1} ds); DHA = dehydrogenase (μg TTF g^{-1} h^{-1} ds); FDA = fluorescein diacetate hydrolase (μg fluorescein g^{-1} ds); CAT = catalase (O2%/3 min/g); MBC = microbial biomass carbon (μg C g^{-1} fs). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Tukey's test, $p \leq 0.05$).

	T0	CTR	NPK	HM	RecOrgFert PLUS
WC	8.25 ^{c} ± 0.91	10.1 ^{b} ± 0.98	14.2 ^{a} ± 1.1	13.4 ^{a} ± 1.1	14.5 ^{a} ± 0.97
pH	8.16 ^{a} ± 0.09	8.30 ^{a} ± 0.1	8.04 ^{b} ± 0.1	8.25 ^{a} ± 0.09	7.5 ^{c} ± 0.1
EC	0.10 ^{c} ± 0.09	0.40 ^{b} ± 0.09	1.08 ^{a} ± 0.1	1.22 ^{a} ± 0.08	1.16 ^{a} ± 0.08
OC	1.13 ^{d} ± 0.18	1.17 ^{d} ± 0.18	2.3 ^{c} ± 0.2	2.54 ^{b} ± 0.18	3.37 ^{a} ± 0.17
TN	1.09 ^{a} ± 0.02	0.11 ^{d} ± 0.02	0.14 ^{c} ± 0.01	0.162 ^{b} ± 0.01	0.194 ^{b} ± 0.01
C/N	1.04 ^{c} ± 1	10.6 ^{b} ± 0.9	16.5 ^{a} ± 1	15.7 ^{a} ± 0.9	17.4 ^{a} ± 1
OM	1.95 ^{d} ± 0.28	2.02 ^{d} ± 0.35	3.99 ^{c} ± 0.3	4.39 ^{b} ± 0.26	5.8 ^{a} ± 0.32
WSP	23.3 ^{c} ± 1.7	26.7 ^{b} ± 2.0	27.5 ^{b} ± 1.6	33.4 ^{a} ± 1.7	35.9 ^{a} ± 2.0
CEC	15.0 ^{b} ± 1	16.5 ^{a} ± 1	17.1 ^{a} ± 0.98	17.3 ^{a} ± 0.97	17.1 ^{a} ± 0.85
DHA	1.4 ^{b} ± 0.28	1.1 ^{b} ± 0.29	2.0 ^{a} ± 0.3	1.6 ^{b} ± 0.3	2.4 ^{a} ± 0.25
FDA	13.3 ^{b} ± 0.51	14.3 ^{b} ± 0.48	15.3 ^{a} ± 0.46	15.4 ^{a} ± 0.53	16.3 ^{a} ± 0.55
CAT	1.3 ^{c} ± 0.33	3.3 ^{a} ± 0.27	3.2 ^{a} ± 0.29	2.1 ^{b} ± 0.31	2.2 ^{b} ± 0.33
MBC	205 ^{c} ± 17.6	218 ^{c} ± 19.1	684 ^{b} ± 14.5	764 ^{a} ± 14.2	791 ^{a} ± 21.1

A similar trend was observed for total nitrogen (TN), which was highest in soils treated with RecOrgFert PLUS (0.194%), followed by horse manure (0.162%) and NPK (0.14%). The control soil had the lowest TN value (0.11%). Consequently, the C/N ratio was significantly influenced by treatments. The highest ratio (17.4) was observed in RecOrgFert PLUS-treated soil, suggesting a slower decomposition rate and potential long-term benefits for organic matter stability.

PCA analysis of the chemical and physical soil properties (Figure 1) treated with different fertilizers clearly showed that RecOrgFert PLUS generated a distinctive chemical profile that set it apart from all other treatments. In quadrant 1, RecOrgFert PLUS showed a strong correlation with organic matter (OM) and CEC; NPK showed a light correlation with OM, HM correlated lightly with OM and CEC. In quadrant 2, NPK correlated with pH. In quadrant 3, a light correlation was observed between CTR and total nitrogen. In quadrant 4, a light correlation was observed between RecOrgFert PLUS and C/N, EC and water content.

Cation and anion concentrations varied across the treatments (Table 2). Sodium and potassium were the highest in soils treated with RecOrgFert PLUS, NPK, and HM. Calcium and magnesium levels remained unchanged compared to the control. Among the anions, SO_4^{2-} was the highest in soils treated with RecOrgFert PLUS. NO_{3^-} was detected only in soil treated with HM; PO_4^{3-}, although present in low amounts, was significantly higher in HM-treated soil compared to the others.

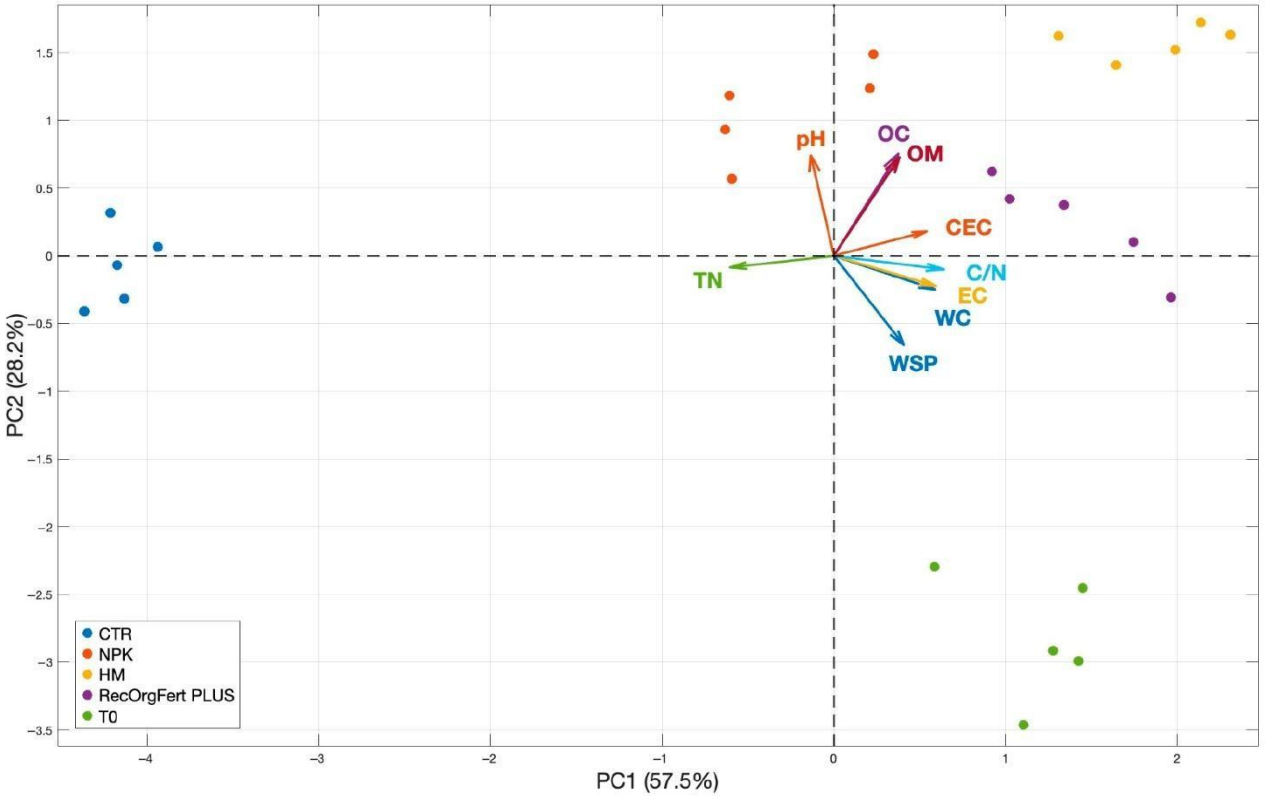


Figure 1. Principal Component Analysis (PCA) of the soil's physical and chemical properties under different fertilization treatments. CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. WC = water content; pH= potential of hydrogen; EC = electrical conductivity; OC = organic carbon; TN = total nitrogen; C/N = carbon-nitrogen ratio; OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity.

Table 2. Concentrations of soil cations and anions under different fertilization treatments. T0 = time zero; CTR (control) soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. Cations and anions concentrations (mg/L): Na^+ (sodium), K^+ (potassium), Ca^{2+} (calcium), Mg^{2+} (magnesium), Cl^- (chloride), NO_2^- (nitrite), NO_3^- (nitrate), PO_4^{3-} (phosphate), SO_4^{2-} (sulphate). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Tukey's test $p \leq 0.05$).

Cations and Anions	T0	CTR	NPK	HM	RecOrgFert PLUS
Na^+	0.012 ^{b} ± 0.0003	0.016 ^{b} ± 0.0003	0.025 ^{a} ± 0.0003	0.024 ^{a} ± 0.0002	0.026 ^{a} ± 0.0003
K^+	0.013 ^{b} ± 0.0003	0.012 ^{b} ± 0.0003	0.027 ^{a} ± 0.0003	0.025 ^{a} ± 0.0002	0.030 ^{a} ± 0.0003
Ca^{2+}	0.02 ^{a} ± 0.005	0.03 ^{a} ± 0.005	0.03 ^{a} ± 0.005	0.03 ^{a} ± 0.004	0.03 ^{a} ± 0.005
Mg^{2+}	7.6 ^{b} ± 0.82	9.3 ^{a} ± 0.79	9.3 ^{a} ± 0.85	9.3 ^{a} ± 0.83	9.7 ^{a} ± 0.78
Cl^-	0.55 ^{b} ± 0.14	0.68 ^{a} ± 0.16	0.66 ^{a} ± 0.15	0.67 ^{a} ± 0.13	0.66 ^{a} ± 0.14
NO^{2-}	nd	0.001 ^{b} ± 0.0001	0.007 ^{a} ± 0.0001	0.001 ^{b} ± 0.0001	0.007 ^{a} ± 0.0001
NO^{3-}	nd	nd	nd	0.026 ^{a} ± 0.0105	nd
PO_4^{3-}	nd	nd	0.002 ^{b} ± 0.0001	0.006 ^{a} ± 0.0001	0.002 ^{b} ± 0.0001
SO_4^{2-}	0.001 ^{c} ± 0.0003	0.001 ^{c} ± 0.0003	0.002 ^{b} ± 0.0003	0.002 ^{b} ± 0.0002	0.003 ^{a} ± 0.0001

3.2. Biological Properties of the Soil

Soil enzymatic activities were also significantly affected by the type of fertilizer applied. DHA, a key indicator of microbial oxidative metabolism, was the highest in RecOrgFert PLUS (2.4 µg TTF g^{-1} h^{-1}), followed by NPK (2.0 µg TTF g^{-1} h^{-1}), HM (1.6 µg TTF g^{-1} h^{-1}), and CTR (1.1 µg TTF g^{-1} h^{-1}). Fluorescein diacetate hydrolase activity showed a similar trend, with RecOrgFert PLUS achieving the highest activity (16.3 µg fluorescein g^{-1}). Catalase activity was the highest in the control and NPK-treated soils. MBC was significantly improved by organic fertilization: RecOrgFert PLUS 791 µg C g^{-1} fs; HM 764 µg C g^{-1} fs; NPK 684 µg C g^{-1} fs and CTR 218 µg C g^{-1} fs (Table 1). PCA of biological properties (Figure 2) revealed that RecOrgFert had a distinctive impact on soil enzyme activity. Its position in quadrant 4 showed a strongly associated correlation with microbial biomass carbon (MBC) and DHA.

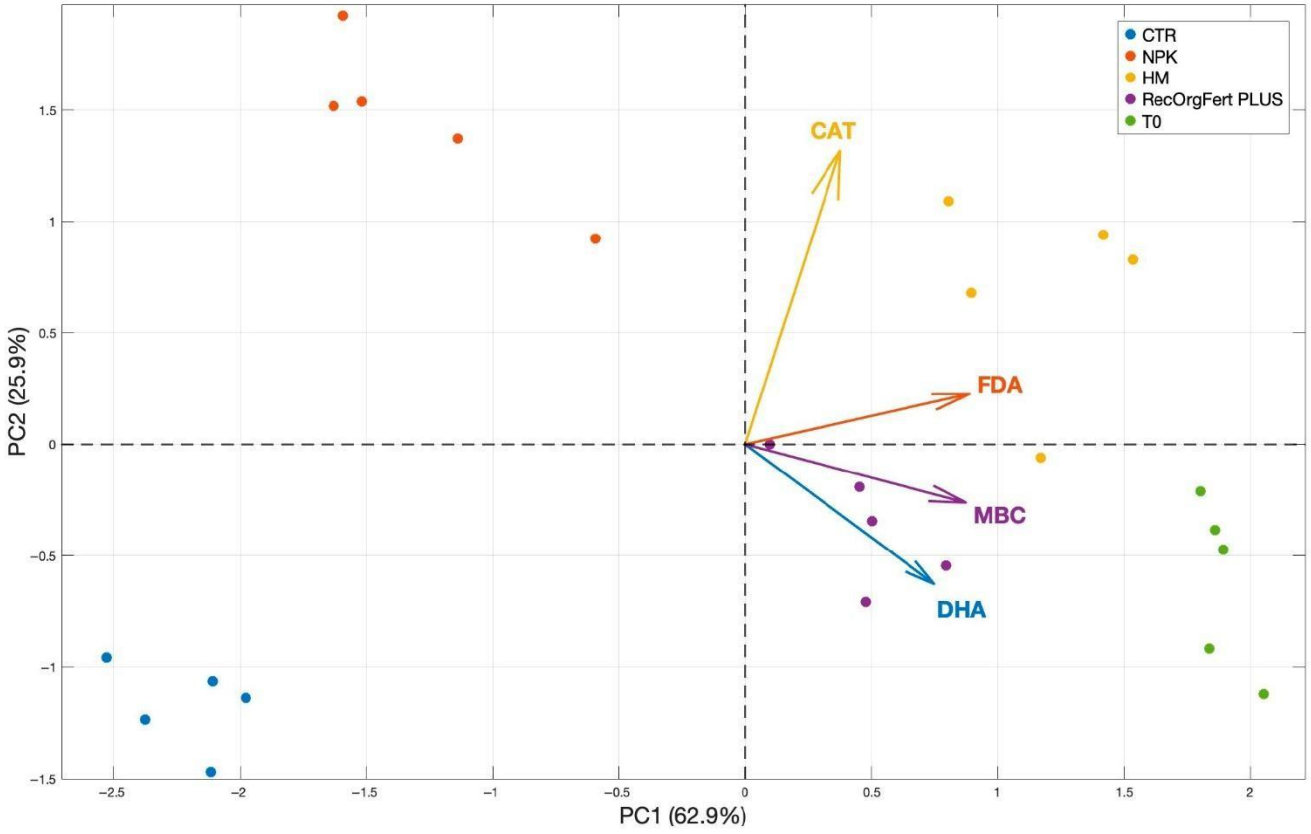


Figure 2. Principal component analysis (PCA) analysis of the soil's biochemical properties under different treatments: CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; CAT = catalase; MBC = microbial biomass carbon.

Pearson's correlation analysis (Figure 3) revealed two distinct functional networks within the soil system. The first encompasses parameters associated with organic fertility—such as organic matter content, water retention, carbon storage, and microbial biomass—which showed strong positive correlations, indicating synergistic interactions.



Figure 3. Pearson correlation matrix illustrating the relationships between biochemical, chemical and physical soil parameters. The correlation coefficients range from −1 (strong negative correlation, in dark blue) to +1 (strong positive correlation, in yellow), with the color scale visible on the right. WC = water content; pH= potential of hydrogen; EC = electrical conductivity; OC = organic carbon; TN = total nitrogen; C/N = carbon–nitrogen ratio; OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; CAT = catalase; MBC = microbial biomass carbon. In the correlation analysis graph, the yellow color indicates statistical significance level at $p < 0.001$; the green color indicates statistical significance at $p < 0.01$; and the blue color and its tonality indicates statistical significance at $p < 0.05$.

3.3. QBS-ar Index

Soil biodiversity, assessed through QBS-ar values (Figure 4), was significantly influenced by the type of fertilizer. Soils treated with RecOrgFert PLUS showed the highest QBS-ar scores, indicating a biologically more active and structured soil community compared to NPK and HM. The soil treated with NPK showed the lowest biodiversity index, inducing the least favorable environment for soil fauna among the fertilizers tested.

The QBS-ar index, which reflects the diversity and adaptation level of soil microarthropods, showed significant differences among treatments (Figure 4). The RecOrgFert PLUS treatment recorded the highest QBS-ar value (53), indicating a more biologically active and structured soil. This suggests that the recycled organic fertilizer, enriched with orange waste and S, creates favorable conditions for edaphic fauna.

HM treatment also enhanced biodiversity (45), while the control showed an intermediate value (38). In contrast, NPK treatment resulted in the lowest QBS-ar value (25), indicating a negative effect of synthetic fertilization on soil fauna, likely due to limited organic inputs and possible chemical imbalances.

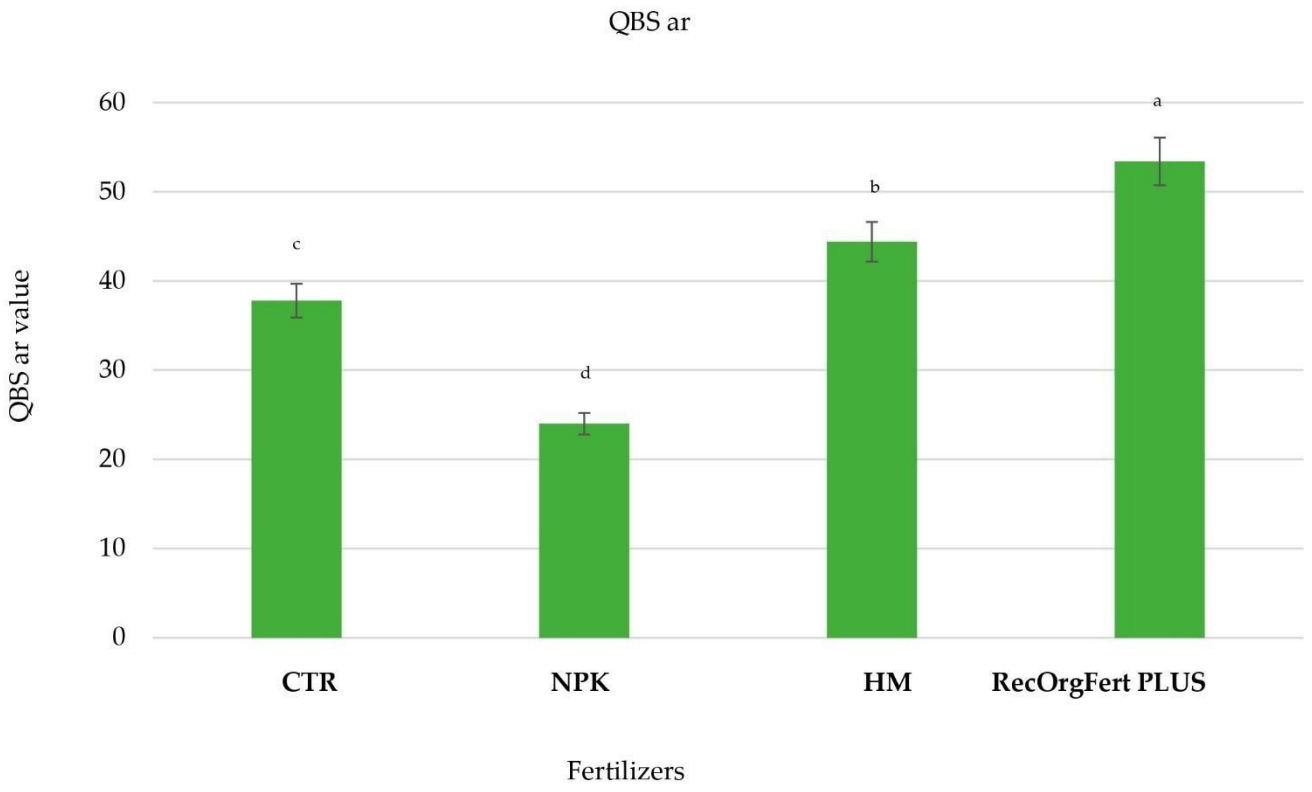


Figure 4. Soil Biological Quality (QBS-ar Index) under different fertilization treatments. CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. Histograms represent QBS-ar index values. Data are the means of three replicates ± standard deviation. Different letters indicate significant differences (Tukey's test $p \leq 0.05$).

3.4. Broccoli Quality

Fertilization strategies significantly influenced the biochemical composition of broccoli (Table 3). Water content was not significantly affected by the treatments. Total phenolic content was the highest in broccoli treated with RecOrgFert PLUS (48.87 mg GAE g^{-1}), followed by HM (43.86 mg GAE g^{-1}), NPK (43.35 mg GAE g^{-1}), and CTR (42.09 mg GAE g^{-1}). Total flavonoid content was significantly higher in broccoli treated with RecOrgFert PLUS and HM, compared to CTR and NPK. DPPH antioxidant activity did not vary significantly among the treatments. However, $ABTS^{+}$ and TAC antioxidant capacities were significantly higher in broccoli treated with RecOrgFert PLUS and HM respect to CTR and NPK. Vitamin C content increased significantly compared to the control, reaching the highest levels in RecOrgFert PLUS (51.93 mg ASA 100 g^{-1}) and HM (50.97 mg ASA 100 g^{-1}) treatments. Vitamin E was higher in all fertilized treatments than in CTR. Total proteins did not change among the treatments. Total carbohydrate content was also the highest in broccoli treated with RecOrgFert PLUS and HM.

PCA analysis of the nutraceutical properties of broccoli grown on soil treated with different fertilizers (Figure 5) clearly showed that RecOrgFert PLUS generated a distinctive chemical profile that differentiated it from all other treatments. In the first quadrant, RecOrgFert PLUS showed a strong correlation with DPPH and total phenolics; NPK showed a strong correlation with total phenolics. In the fourth quadrant, a slight correlation was observed between RecOrgFert PLUS and Vitamin E, TAC, $ABTS^{+}$, Vitamin C; in the same quadrant a weak correlation was observed between Horse Manure and Vitamin C, total flavonoids, total carbohydrates; NPK in the fourth quadrant had a strong correlation with total proteins.

Table 3. Analysis of broccoli grown in the soils of Condofuri: CTR=control soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. WC = water content (%); DW = dry weight (%); TP = total phenols (mg GAE g^{-1}); TF = total flavonoids (mg QE g^{-1}); DPPH = 2,2-difenil-1-picrilidrazile (% inhibition); $ABTS^+$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato (µM Trolox g^{-1}); TAC = total antioxidant capacity (mg α-tocopherol 100 g^{-1}); Vit C = vitamin C (mg ascorbic acid 100 g^{-1}); Vit E = vitamin E (mg α-tocopherol 100 g^{-1}), TPRO = total protein (mg Bovine Serum Albumin (BSA) g^{-1}), TCARB = total carbohydrates (mg glucose g^{-1}). Data are the means of three replicates ± standard error. Different letters in the same row indicate significant differences (Tukey's test, $p \leq 0.05$).

	CTR	NPK	HM	RecOrgFert PLUS
WC	90.65 ^{a} ± 2.1	89.14 ^{a} ± 2.5	87.24 ^{a} ± 2.4	86.32 ^{a} ± 2.6
DW	9.35 ^{a} ± 0.9	10.86 ^{b} ± 0.5	12.76 ^{b} ± 0.6	13.68 ^{c} ± 0.4
TP	42.09 ^{b} ± 1.8	43.35 ^{b} ± 1.9	43.86 ^{b} ± 1.9	48.87 ^{a} ± 2.1
TF	5.29 ^{b} ± 0.3	5.76 ^{b} ± 0.3	6.28 ^{a} ± 0.4	6.29 ^{a} ± 0.4
DPPH	23.25 ^{a} ± 1.2	23.54 ^{a} ± 1.2	23.37 ^{a} ± 1.2	24.85 ^{a} ± 1.3
$ABTS^+$	3.39 ^{c} ± 0.2	4.37 ^{b} ± 0.2	5.26 ^{a} ± 0.3	5.29 ^{a} ± 0.3
TAC	4.43 ^{c} ± 0.3	5.62 ^{b} ± 0.3	6.98 ^{a} ± 0.4	7.13 ^{a} ± 0.4
Vit C	18.98 ^{c} ± 1.1	42.17 ^{b} ± 2.3	50.97 ^{a} ± 2.8	51.93 ^{a} ± 2.9
Vit E	1.52 ^{b} ± 0.1	1.87 ^{a} ± 0.1	1.89 ^{a} ± 0.1	1.93 ^{a} ± 0.1
TPRO	80.21 ^{a} ± 3.2	83.05 ^{a} ± 3.3	82.42 ^{a} ± 3.3	82.45 ^{a} ± 3.3
TCARB	124.07 ^{c} ± 12.4	220.65 ^{b} ± 12.1	237.93 ^{a} ± 12.8	239.93 ^{a} ± 13.0

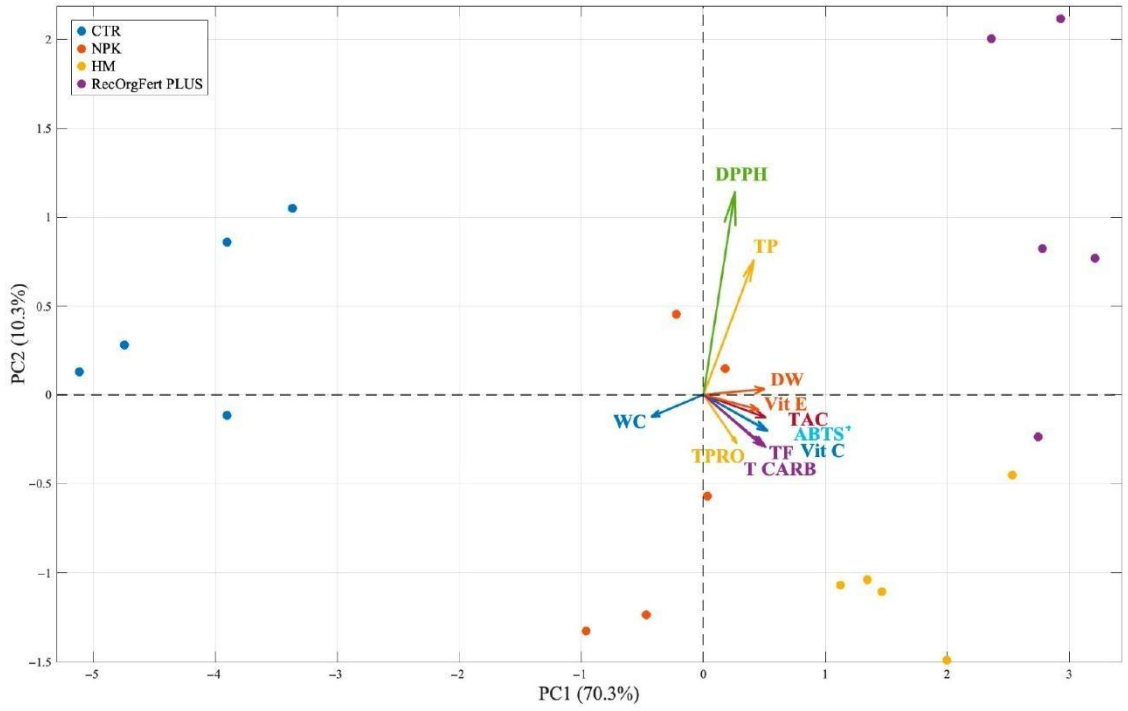


Figure 5. Principal Component Analysis (PCA) of the nutraceutical properties of broccoli cultivated in soils under different fertilization treatments. Treatments include CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. WC = water content; DW = dry weight; TP = total phenols; TF = total flavonoids; DPPH = 2,2-difenil-1-picrilidrazile; $ABTS^+$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit C = vitamin C; Vit E = vitamin E; TPRO = total protein; TCARB = total carbohydrates.

Pearson's correlation analysis (Figure 6) revealed a well-defined network of interactions among variables related to antioxidant capacity. Strong positive correlations were observed between ABTS$^+$, total antioxidant capacity (TAC), vitamin C, and total flavonoids (TF), suggesting that these parameters were functionally interconnected and likely reflected a shared underlying antioxidant mechanism. Similarly, total carbohydrates (TCARB) showed strong associations with ABTS$^+$, vitamin C, TAC, and TF, indicating a possible supportive role of carbohydrates in the stabilization, transport, or accumulation of antioxidant compounds.



Figure 6. Pearson correlation matrix of broccoli quality parameters. The color gradient indicates the strength and direction of the correlations, with positive correlations shown in blue and negative correlations in red. Strong correlations highlight potential interdependencies among quality traits influenced by different soil fertilization treatments. WC = water content; DW = dry weight; TP = total phenols; TF = total flavonoids; DPPH = 2,2-difenil-1-picrilidrazile; ABTS$^+$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit C = vitamin C; Vit E = vitamin E; TPRO = total protein; TCARB = total carbohydrates. In the correlation analysis graph, the yellow color indicates statistical significance level at $p < 0.001$; the green color indicates statistical significance at $p < 0.01$; and the blue color and its tonality indicates statistical significance at $p < 0.05$.

3.5. Correlations of Soil Parameters and Broccoli Quality Traits

Pearson correlation matrix between soil parameters and broccoli quality traits (Figure 7) revealed that fluorescein diacetate hydrolysis exhibited consistently strong positive correlations with all broccoli quality parameters (all values > 0.8812), particularly with total phenols (TP, 0.9180), ABTS$^+$ radical scavenging activity (0.8918), and vitamin E (0.8982). Soil organic matter (OM) showed high correlations with total antioxidant capacity (TAC, 0.9286) and total flavonoids (TF, 0.9183).

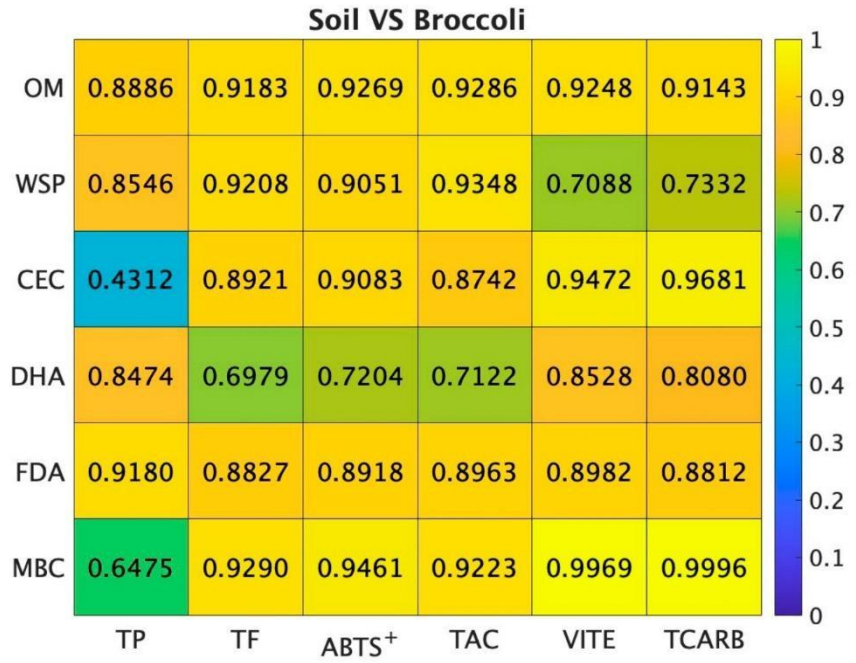


Figure 7. Pearson correlations matrix between soil quality and broccoli quality. The color gradient indicates the strength and direction of the correlations, with positive correlations shown in blue and negative correlations in red. OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; MBC = microbial biomass carbon; TP = total phenols; TF = total flavonoids; $ABTS^+$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit E = vitamin E; TCARB = total carbohydrates. In the correlation analysis graph, the yellow color indicates statistical significance level at $p < 0.001$; the green color indicates statistical significance at $p < 0.01$; and the blue color and its tonality indicates statistical significance at $p < 0.05$.

Microbial biomass carbon (MBC) had very high correlations, with vitamin E (0.9969) and total carbohydrate (TCARB, 0.9996) and moderate correlation with TP (0.6475). MBC emerged as particularly relevant for vitamin E and carbon accumulation. Water-soluble phenols were strongly correlated with TAC (0.9348) and $ABTS^+$ (0.9051). Cation exchange capacity displayed moderate to strong correlations with most quality parameters, showing the strongest correlations with vitamin E (0.9472) and TCARB (0.9681). Dehydrogenase activity (DHA) weakly correlated with TF (0.6979) and TAC (0.7122), and moderately with TP and vitamin E.

When analyzing the correlations between soil parameters and broccoli quality by fertilization treatments (Figure 8), it emerged that OM showed modest correlations with TP (0.3015) and TCARB (0.5606). WSP showed a strong negative correlation with TF (−0.9586) and a positive correlation with vitamin E (0.7410). CEC was moderately correlated with $ABTS^+$ (0.7746). DHA was strongly correlated with TF (0.8660) and vitamin E (0.9028), but showed a marked negative correlation with TCARB (−0.9683). FDA exhibited weak or negative correlations with most parameters (TF: −0.7256; TCARB: −0.6831). MBC showed only moderate positive correlations with TP (0.5774) and $ABTS^+$ (0.3333), and negative correlations with TF (−0.8568) and TAC (−0.5774). Overall, in the absence of fertilization, correlations between soil quality indices and broccoli nutritional and nutraceutical quality were weaker and less consistent.

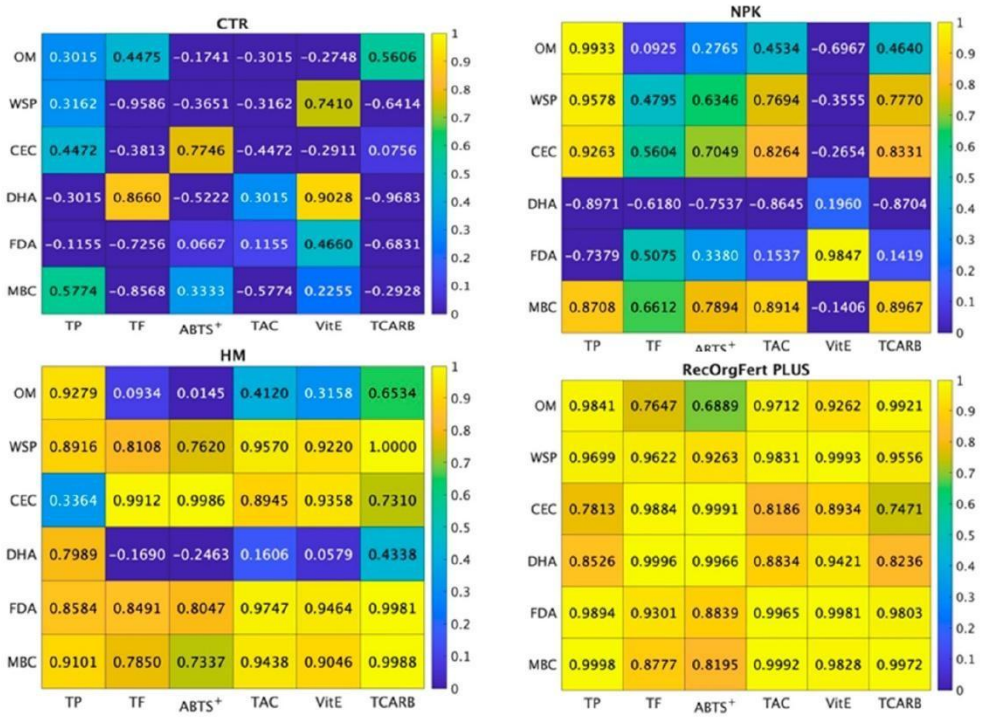


Figure 8. Pearson correlations matrix between soil quality and broccoli quality treated with different fertilizers: CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert PLUS = S bentonite + orange residue. The color gradient indicates the strength and direction of the correlations, with positive correlations shown in blue and negative correlations in red. OM = organic matter; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; MBC = microbial biomass carbon; TP = total phenols; TF = total flavonoids; $ABTS^+$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit E = vitamin E; TCARB = total carbohydrates. In the correlation analysis graph, the yellow color indicates statistical significance level at $p < 0.001$; the green color indicates statistical significance at $p < 0.01$; and the blue color and its tonality indicates statistical significance at $p < 0.05$.

The application of horse manure substantially enhanced correlations between biological soil parameters and antioxidant components of broccoli. SOM showed strong correlations with TP (0.9279) and TCARB (0.6534). WSP displayed high correlations with all quality traits, particularly TAC (0.9570) and TCARB (1.0000). CEC showed very high correlations with TF (0.9912), $ABTS^+$ (0.9986), and Vitamin E (0.9358). DHA generally showed low or negative correlations, with TF (−0.1690) and $ABTS^+$ (−0.2463). FDA was strongly correlated with TAC (0.9747), vitamin E (0.9464), and TCARB (0.9981). MBC was highly correlated with TP (0.9101), TAC (0.9438), and TCARB (0.9988). Overall, WSP, CEC, FDA, and MBC were the best predictors of broccoli nutritional and nutraceutical quality, especially total carbohydrate content, TAC, and antioxidant compounds.

Under NPK treatment, strong positive correlations were observed between OM and TP (0.9933), WSP and TP (0.9578), CEC and TP (0.9263), MBC and TAC (0.8914), and MBC and TCARB (0.8967), indicating a direct influence of soil quality on total antioxidants and carbohydrate content. DHA showed strong negative correlations with TP (−0.8971), TAC (−0.8645), and TCARB (−0.8704), suggesting a potentially adverse effect of dehydrogenase activity on nutritional and nutraceutical quality. A high correlation between FDA and vitamin E (0.9847) indicates a strong association between enzymatic activity and vitamin E content. These results suggest that chemical and biochemical soil properties significantly influence the nutritional and nutraceutical quality of broccoli under NPK fertilization.

Pearson correlations between soil properties and broccoli quality under RecOrgFert PLUS treatment were very high across nearly all combinations, indicating a strong association between soil quality and nutritional and nutraceutical quality. Specifically, MBC was highly correlated with TP (0.9998), TAC (0.9992), vitamin E (0.9828), and TCARB (0.9972). FDA showed strong correlations with vitamin E (0.9981), TAC (0.9965), and TCARB (0.9803). DHA exhibited extremely high correlations with TF (0.9996) and $ABTS^+$ (0.9966), in contrast to the negative correlations observed under NPK. WSP and CEC also showed high values, with WSP–vitamin E at 0.9993 and CEC– $ABTS^+$ at 0. These findings suggest that RecOrgFert PLUS created a pronounced synergistic effect between soil quality and the nutritional and nutraceutical profile of broccoli, outperforming other treatments.

4. Discussion

4.1. Soil Physicochemical Properties

This study evidenced that RecOrgFert PLUS led to the highest increases in soil organic carbon (OC), total nitrogen (TN), and microbial biomass carbon (MBC), compared to NPK and HM, demonstrating that its formulation containing elemental S plus organic components was able to contribute to long-term carbon sequestration in the soil and to improve nutrient availability, as already demonstrated by other authors [5] for different matrices containing organic components. Compared to other sustainable fertilizers such as compost and vermicompost, which generally increase soil microbial biomass and enzymatic activity [38–40], RecOrgFert PLUS has demonstrated equal or even greater improvements in soil microbial health. Unlike compost, which requires long maturation periods to prevent phytotoxicity and ensure microbial stability [41], RecOrgFert PLUS provides readily available nutrients, promoting a rapid and efficient microbial response. Furthermore, due to its controlled formulation, it avoids the uncontrolled ammonia emissions and nutrient dilution often observed in low-quality composts [11,42,43].

4.2. Biological Properties of the Soil

Any changes in soil MBC have a significant impact on soil carbon, nitrogen, phosphorus, plant species and the dynamics of the terrestrial ecosystems. MBC can be used as the early prediction index of soil quality and the change of soil total organic matter. Soil enzyme activities reflect the rate of soil nutrient cycling and utilization and may be an index of soil biodiversity, productivity and potential microbial activity. In the present study, soil enzyme activities, particularly dehydrogenase and fluorescein diacetate hydrolase, significantly increased in soils treated with RecOrgFert PLUS. These enzymes are widely recognized as indicators of soil microbial activity and biochemical fertility [44,45]. The observed increase in enzymatic activity suggested that RecOrgFert PLUS is able to provide a favorable environment for microbial communities, likely due to its organic composition and slow nutrient release [43]. The lower level of catalase activity supports this hypothesis, suggesting a reduction of soil stress levels which is in line with previous results of Song et al. [46], who reported that catalase can serve as a biochemical marker for providing insights into the community responses to exogenous stress.

Furthermore, it is interesting to note that catalase activity was significantly higher in the NPK treatment than in the others, particularly compared to RecOrgFert PLUS, indicating a higher presence of oxidative stress in soils treated with synthetic fertilizers, as suggested by studies that associate increased catalase activity with the need to detoxify free radicals, generated by oxidative reactions induced by intensive chemical inputs [46]. Conversely, the lower CAT values found in the RecOrgFert PLUS treatment may reflect a more stable microbial environment that is less subject to environmental stress, confirming the hypothesis of a better ecological balance in the soil.

Like biochar and vermicompost, which improve soil structure and faunal diversity [47], RecOrgFert PLUS supported a more structured soil fauna community. The higher QBS-ar index observed with RecOrgFert suggest it is at least as effective as biochar and avoids potential antagonistic interactions noted in combined amendments [48].

PCA analysis of the chemical and physical soil properties treated with different fertilizers clearly showed that RecOrgFert PLUS generated a distinctive chemical profile that set it apart from all other treatments. The distribution of RecOrgFert PLUS variables in the quadrants evidences a greater dispersion, suggesting more heterogeneous effects of this treatment, with unique chemical changes in soil. These effects are likely due to its specific formulation, which synergistically combines organic and mineral components. The distance from control samples further confirms the effectiveness of the treatment in modifying soil chemistry, while the tight clustering of the treated samples demonstrates the consistency and reproducibility of its effects.

Similarly, the analysis of biological properties indicates that RecOrgFert has a distinct influence on soil enzyme activity. It is closely associated with increased levels of microbial biomass carbon (MBC) and dehydrogenase activity (DHA), suggesting that this fertilizer effectively promotes the growth and functional activity of the soil microbial community. The clear separation from NPK and HM treatments further indicates that RecOrgFert PLUS creates a unique enzymatic profile, likely due to its ability to provide specific substrates that enhance microbial activity. Microbial biomass is a key indicator of soil vitality, as it represents the active fraction of organic matter responsible for the decomposition of organic matter and the release of essential nutrients for plants, thus contributing to soil fertility and health [49].

Pearson's correlation analysis revealed a tightly interconnected group of soil properties associated with organic fertility. The strong positive relationships among these variables suggest that soil amendments can trigger coordinated improvements across key biological and physicochemical functions, reflecting the activation of integrated soil processes. These relationships suggest that fertilizer treatments have activated integrated biogeochemical processes, whereby the improvement of one parameter promotes enhancements in the others. This is supported by recent studies demonstrating that the application of organic fertilizers, such as cow manure and straw residues, significantly enhances soil organic carbon pools and microbial activity, leading to improved soil fertility and sustainability [50].

On the other hand, the consistent negative correlation of pH with organic and enzymatic parameters suggests that the fertilizers caused a slight acidification of the soil. Rather than being detrimental, this acidification reflects the intensification of organic matter decomposition and nutrient mineralization, which naturally release organic acids. The resulting mildly acidic environment appears to stimulate microbial activity—particularly of dehydrogenase, a key enzyme in microbial metabolism—as confirmed by its strong negative correlation with pH [4].

In line with our results, Liu et al., [42] demonstrated that organic fertilizers improved microbial diversity and functionality, enhancing soil health compared to conventional fertilization. The increased concentrations of Na^+, K^+, and SO_4^{2-} in soils treated with RecOrgFert PLUS indicated a better nutrient supply compared to the control. These results are consistent with studies highlighting that fertilizers containing organic components contribute to an increased cation exchange capacity (CEC) of the soil, thus improving nutrient retention and reducing losses due to leaching [5]. Interestingly, NO_{3^-} was detected only in the horse manure treatment, suggesting a different nitrogen mineralization pattern. Synthetic fertilizers, such as NPK, tend to release nitrogen rapidly, leading to potential leaching [11]. In contrast, RecOrgFert PLUS releasing nutrients more gradually could improve nitrogen use efficiency, reducing nitrogen environmental impact [51].

4.3. QBS-ar Index

The increase in biodiversity observed in soils treated with RecOrgFert PLUS, as indicated by QBS-ar values, supports the finding that RecOrgFert Plus created a more favorable habitat for soil fauna. Several studies reported similar findings, demonstrating that organic fertilization or fertilizers with organic components improved soil biological quality by increasing microbial biomass, enzymatic activity, and macrofauna diversity [5,43]. Soil biodiversity is a crucial indicator of soil resilience and functionality, as it contributes to organic matter decomposition, nutrient cycling, and the maintenance of soil structure [52]. The improvement in the QBS-ar index in soils treated with RecOrgFert PLUS suggests that waste-based fertilizers can play a key role in promoting more stable and self-sufficient soil ecosystems, a feature that is increasingly relevant in sustainable agriculture.

4.4. Broccoli Quality

The different types of fertilizers used significantly influenced the biochemical composition of broccoli: plants treated with RecOrgFert PLUS showed the highest levels of total phenols, flavonoids, and vitamin C. In particular, the increase in total phenolic content observed in broccoli treated with RecOrgFert PLUS aligns with studies demonstrating that organic amendments can improve the antioxidant profile of crops [53]. This effect can be attributed to the improved soil organic matter content and microbial activity, which facilitate the uptake of micronutrients essential for phenolic biosynthesis [43]. Notably, the significantly higher vitamin C content in RecOrgFert PLUS- and horse manure-treated broccoli (compared to synthetic fertilization, NPK) is also worth mentioning. Organic fertilization has been shown to enhance ascorbic acid accumulation in vegetables due to lower nitrogen availability, which promotes secondary metabolite synthesis rather than excessive vegetative growth [54]. This is in line with our results, suggesting that waste-based fertilizers may offer advantages in improving both yield and the nutritional and nutraceutical quality of broccoli. Similarly, the increase in total protein and carbohydrate content in broccoli treated with RecOrgFert PLUS supports previous evidence that organic fertilization enhances the accumulation of macronutrients in crops [51]. The increase in protein content could be related to better nitrogen absorption efficiency, while the increase in carbohydrate levels could be linked to greater microbial interaction in the soil, which has been shown to promote photosynthetic efficiency and carbon fixation [42].

The results of the PCA highlighted how the choice of fertilizer profoundly affected not only plant growth but also the nutritional and nutraceutical quality of broccoli. The distinct placement of RecOrgFert PLUS in the upper right quadrant, correlated with higher contents of bioactive compounds, suggests that this fertilizer could specifically stimulate the metabolic pathways responsible for the synthesis of flavonoids and other phenolic compounds in broccoli. These compounds are known for their antioxidant properties and potential human health benefits. The clear separation between RecOrgFert PLUS-treated and control samples indicates that intervention with this biofertilizer produces a substantial improvement in nutritional and nutraceutical quality compared with standard growing conditions. This suggests that RecOrgFert PLUS could be a significant innovation to produce vegetables with enhanced nutritional profiles. The intermediate placement of samples treated with horse manure indicates that this conventional organic fertilizer offers some nutritional benefits over the control but does not achieve the quality levels obtained with RecOrgFert PLUS. This observation is particularly relevant to organic farming systems seeking sustainable alternatives to synthetic fertilizers. The variability observed in NPK samples might reflect a less consistent plant response to this type of chemical fertilizer, suggesting that immediate availability of inorganic nutrients may not necessarily translate into uniform improvement in nutritional and nutraceutical quality. The positive correlation

between several nutritional and nutraceutical parameters (total phenols, DPPH, VITE, TAC and $ABTS^+$) in RecOrgFert PLUS-treated samples suggests that this fertilizer may activate complex physiological mechanisms that simultaneously improve several aspects of broccoli nutritional and nutraceutical quality, rather than affecting individual isolated parameters. These results have important implications for agricultural practices oriented not only to productivity but also to nutritional and nutraceutical quality of food, highlighting how RecOrgFert PLUS may represent an innovative solution for growing brassicas with enhanced nutraceutical properties.

4.5. Correlations of Soil Parameters and Broccoli Quality Traits

Correlation matrix analysis reveals significant relationships between the different biochemical parameters examined. The high concordance between the different antioxidant assays ($ABTS^+$, TAC, DPPH) confirms the validity of the measurements, while the slight variations in their correlations highlight the specificity of each method towards antioxidants. The extraordinary correlation between vitamin C and total carbohydrates (0.9732) suggests potential metabolic or structural relationships between these components. Carbohydrates, in fact, act as precursors for vitamin C synthesis, primarily through the Smirnoff–Wheeler pathway. Specifically, D-glucose and D-mannose, both simple sugars, are converted into compounds like GDP-D-mannose, L-galactose, and L-galactono-1,4-lactone, which are then used to produce vitamin C [55]. The differential correlations of phenolic compounds with the different antioxidant assays (strongly correlated with DPPH activity and moderately correlated with TAC, $ABTS^+$ and Vitamin C) indicate an integrated and synergistic antioxidant system in the sample analyzed, where vitamin C, phenolic compounds and potentially some carbohydrate fractions collectively contribute to the total antioxidant activity through complementary mechanisms. Flavonoids correlated strongly with $ABTS^+$, TAC and Vitamin C, indicating that crops with higher flavonoid levels may also exhibit greater antioxidant activity. Pearson correlation analysis revealed that FDA, OM, and MBC were the most influential soil properties associated with broccoli quality. These parameters showed strong and consistent correlations with key broccoli traits, including total phenols (TP), total flavonoids (TF), antioxidant activity ($ABTS^+$, TAC), vitamin E, and total carbohydrate content. Among these, FDA emerged as the primary indicator, reflecting high microbial activity and robust associations with all broccoli quality parameters. Similarly, OM contributed significantly to nutrient availability and soil structure, enhancing antioxidant and nutrient levels in broccoli. MBC particularly influenced vitamin E content and carbohydrate accumulation. Conversely, CEC and DHA showed more variable correlations, with CEC performing poorly for TP and DHA being the most inconsistent overall. WSP played a more targeted role, primarily benefiting antioxidant properties. These results suggest that soil biological activity and organic matter content are key determinants of broccoli's nutritional and nutraceutical quality and may represent strategic targets for improving crop value.

Correlational differences among fertilization treatments highlighted the pivotal role of soil management in shaping the nutritional and nutraceutical attributes of horticultural crops. In the control treatment (CTR), devoid of fertilization, correlations between soil biological parameters and broccoli quality were weak and inconsistent, reflecting limited microbial activity and nutrient availability—typical of unmanaged, low-fertility soils. This is consistent with recent studies showing that the absence of organic or mineral inputs reduces microbial biomass and nutrient-use efficiency in crops [56].

Horse manure application increased the correlations between soil biological parameters and broccoli antioxidant components, indicating its capacity to stimulate microbial processes and provide substrates for secondary metabolite biosynthesis in plants [6,54].

However, performance was not uniformly high, suggesting variability in organic matter mineralization.

Mineral fertilization (NPK) enhanced correlations between soil chemical parameters and broccoli nutrients such as carbohydrates and vitamin E, but correlations with antioxidant parameters were weaker. This suggests that mineral fertilization supports vegetative growth and macronutrient content, but does not effectively stimulate the synthesis of bioactive metabolites such as phenols and flavonoids. These findings align with those of Liu at al. [54] and meta-analyses indicating that mineral nutrition may reduce antioxidant content compared to organic practices [54].

The RecOrgFert PLUS treatment produced the highest, most consistent, and most significant correlations between soil quality and broccoli quality. The coherence and strength of these associations indicate a strong synergistic effect between soil microbiological vitality and the expression of nutraceutical compounds in broccoli.

These findings are supported by recent evidence showing that regenerative organic fertilizers—rich in stable carbon, microbial substrates, and slowly available nutrients—enhance soil microbial biomass, enzymatic activity (e.g., FDA), and crop antioxidant capacity [57]. Furthermore, the high correlations with MBC and OM underscore that increasing soil organic matter and microbial biomass is crucial for improving broccoli's nutritional and nutraceutical quality. This confirms that regenerative approaches are not only sustainable but also beneficial from a nutraceutical standpoint [56].

4.6. General Discussion

Therefore, RecOrgFert PLUS emerged as a high-potential strategic solution for improving the nutritional and nutraceutical quality of horticultural products through the restoration of biological and biochemical soil fertility, outperforming both conventional (NPK) and traditional organic (HM) fertilization approaches.

These results strengthen the growing body of evidence supporting the use of waste-based fertilizers as sustainable alternatives to synthetic fertilization (NPK). The significant improvements in soil health indicators (organic carbon, microbial activity, and enzymatic functions) and crop quality parameters (antioxidants, vitamins, and proteins) suggest that RecOrgFert PLUS has strong potential for application in sustainable agriculture. Given the environmental concerns associated with synthetic fertilizers, such as nitrate leaching and soil degradation [58], waste-based fertilizers represent a promising strategy for improving soil fertility while minimizing ecological impacts.

5. Conclusions

This study highlights the strategic role of waste-based fertilizers, particularly RecOrgFert PLUS, in enhancing soil fertility and crop quality within a sustainable agriculture framework and a circular economy approach. The application of RecOrgFert PLUS contributes to a more biologically active and structured soil environment, indicating improved ecological quality, greater organic matter stability, and optimized nutrient availability—factors essential for long-term agricultural productivity. Broccoli cultivated with RecOrgFert PLUS demonstrated a superior nutritional and nutraceutical profile, with notable increases in total phenols, vitamin C, and protein content compared to other treatments. These improvements are likely linked to enhanced soil biodiversity and beneficial microbe–plant interactions that support nutrient uptake and the synthesis of bioactive, nutraceutical compounds. These findings underscore the potential of waste-based fertilizers to enhance both soil health and the nutritional and nutraceutical quality of crops, contributing to more sustainable food systems. By recycling organic waste into valuable agricultural inputs, this approach aligns with circular economy principles, reducing environmental

impact and strengthening the resilience of agroecosystems. Continued long-term research is recommended to evaluate the effects of RecOrgFert PLUS across different soil types and crop species, aiming to optimize its large-scale adoption and integration into sustainable agricultural practices.

Author Contributions: Conceptualization, A.M.; methodology, F.M.; software, L.S.; validation, L.S., S.B., F.M., M.O. and C.M.; investigation, M.O.; resources, A.M.; data curation, M.O.; writing—original draft preparation, A.M.; writing—review and editing, M.O., L.S. and A.M.; visualization, S.B.; supervision A.M. and M.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Italian Ministry for University and Research (MUR), PNRR Project CN_00000022, "National Research Centre for Agricultural Technologies Agritech" and "Solutions for soil quality assessment and protection" 3.2.1.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

T0	Time zero
CTR	Control, soil without fertilizer
NPK	Nitrogen–phosphorus–potassium
HM	Horse manure
S	Sulphur
WC	Water content
EC	Electrical conductivity
OC	Organic carbon
TN	Total nitrogen
C/N	Carbon–nitrogen ratio
OM	Organic matter
WSP	Water-soluble phenols
CEC	Cation exchange capacity
DHA	Dehydrogenase
FDA	Fluorescein diacetate hydrolase
CAT	Catalase
MBC	Microbial biomass carbon
DW	Dry weight
TP	Total phenols
TF	Total flavonoids
DPPH	2,2-difenil-1-picrilidrazile
$ABTS^{+}$	2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato
TAC	Total antioxidant capacity
Vit C	Vitamin C
Vit E	Vitamin E
TPRO	Total proteins
TCARB	Total carbohydrates
PCA	Principal component analysis

References

1. Tahat, M.; Alananbeh, K.; Othman, Y.; Leskovar, D. Soil Health and Sustainable Agriculture. *Sustainability* **2020**, *12*, 4859. [CrossRef]

2. Lazcano, C.; Boyd, E.; Holmes, G.; Hewavitharana, S.; Pasulka, A.; Ivors, K. The Rhizosphere Microbiome Plays a Role in the Resistance to Soil-Borne Pathogens and Nutrient Uptake of Strawberry Cultivars under Field Conditions. *Sci. Rep.* **2021**, *11*, 3188. [CrossRef]
3. Amin, F.; Jilani, M.I. Environmental, Microbiological and Chemical Implications of Fertilizers Use in Soils: A Review. *Int. J. Chem. Biochem. Sci.* **2024**, *25*, 56–73.
4. Zhang, L.; Zhao, Z.; Jiang, B.; Baoyin, B.; Cui, Z.; Wang, H.; Li, Q.; Cui, J. Effects of Long-Term Application of Nitrogen Fertilizer on Soil Acidification and Biological Properties in China: A Meta-Analysis. *Microorganisms* **2024**, *12*, 1683. [CrossRef]
5. Badagliacca, G.; Testa, G.; La Malfa, S.G.; Cafaro, V.; Lo Presti, E.; Monti, M. Organic Fertilizers and Bio-Waste for Sustainable Soil Management to Support Crops and Control Greenhouse Gas Emissions in Mediterranean Agroecosystems: A Review. *Horticulturae* **2024**, *10*, 427. [CrossRef]
6. Diacono, M.; Montemurro, F. Long-Term Effects of Organic Amendments on Soil Fertility. In *Sustainable Agriculture Volume 2*; Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P., Eds.; Springer Netherlands: Dordrecht, The Netherlands, 2011; pp. 761–786. ISBN 978-94-007-0394-0.
7. Ali, A.; Jabeen, N.; Farruhbek, R.; Chachar, Z.; Laghari, A.A.; Chachar, S.; Ahmed, N.; Ahmed, S.; Yang, Z. Enhancing Nitrogen Use Efficiency in Agriculture by Integrating Agronomic Practices and Genetic Advances. *Front. Plant Sci.* **2025**, *16*, 1543714. [CrossRef]
8. Muscolo, A.; Papalia, T.; Settineri, G.; Mallamaci, C.; Panuccio, M.R. Sulfur Bentonite-Organic-Based Fertilizers as Tool for Improving Bio-Compounds with Antioxidant Activities in Red Onion. *J. Sci. Food Agric.* **2020**, *100*, 785–793. [CrossRef]
9. Zhou, T.; Wang, Z.; Lv, Q.; Zhang, Y.; Tao, S.; Ren, X.; Gao, H.; Gao, Z.; Hu, S. Sulfur Dynamics in Saline Sodic Soils: The Role of Paddy Cultivation and Organic Amendments. *Ecol. Indic.* **2024**, *162*, 112014. [CrossRef]
10. Singh, K.; Singh, R.; Nwosu, N.J.; Omara, P.; Sharma, L.; Dunn, B.L.; Singh, H. Optimizing Nutrient Delivery in Agronomic Crops: A Review of Enhanced Efficiency Fertilizers. *J. Plant Nutr. Soil Sci.* **2025**. [CrossRef]
11. Drinkwater, L.E.; Snapp, S.S. Advancing the Science and Practice of Ecological Nutrient Management for Smallholder Farmers. *Front. Sustain. Food Syst.* **2022**, *6*. [CrossRef]
12. Mahn, A.; Reyes, A. An Overview of Health-Promoting Compounds of Broccoli (Brassica Oleracea Var. Italica) and the Effect of Processing. *Food Sci. Technol. Int.* **2012**, *18*, 503–514. [CrossRef]
13. Shinali, T.S.; Zhang, Y.; Altaf, M.; Nsabiyeze, A.; Han, Z.; Shi, S.; Shang, N. The Valorization of Wastes and Byproducts from Cruciferous Vegetables: A Review on the Potential Utilization of Cabbage, Cauliflower, and Broccoli Byproducts. *Foods* **2024**, *13*, 1163. [CrossRef]
14. Naguib, A.E.-M.M.; El-Baz, F.K.; Salama, Z.A.; Abd El Baky Hanaa, H.; Ali, H.F.; Gaafar, A.A. Enhancement of Phenolics, Flavonoids and Glucosinolates of Broccoli (*Brassica Olaracea*, Var. *Italica*) as Antioxidants in Response to Organic and Bio-Organic Fertilizers. *J. Saudi Soc. Agric. Sci.* **2012**, *11*, 135–142. [CrossRef]
15. Muscolo, A.; Sidari, M.; Settineri, G.; Papalia, T.; Mallamaci, C.; Attinà, E. Influence of Soil Properties on Bioactive Compounds and Antioxidant Capacity of Brassica Rupestris Raf. *J. Soil Sci. Plant Nutr.* **2019**, *19*, 808–815. [CrossRef]
16. FAO. *Agricultural Biodiversity: FAO Multifunctional Character of Agriculture and Land*; FAO: Rome, Italy, 1999.
17. Muscolo, A.; Mallamaci, C.; Settineri, G.; Calamarà, G. Increasing Soil and Crop Productivity by Using Agricultural Wastes Pelletized with Elemental Sulfur and Bentonite. *Agron. J.* **2017**, *109*, 1900–1910. [CrossRef]
18. Muscolo, A.; Romeo, F.; Marra, F.; Mallamaci, C. Recycling Agricultural, Municipal and Industrial Pollutant Wastes into Fertilizers for a Sustainable Healthy Food Production. *J. Environ. Manag.* **2021**, *300*, 113771. [CrossRef]
19. Bouyoucos, G.J. Hydrometer Method Improved for Making Particle Size Analyses of Soils. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
20. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemists (AOAC) International: Rockville, MA, USA, 2005.
21. Mehlich, A. Rapid Determination of Cation and Anion Exchange Properties and pHe of Soils. *J. Assoc. Off. Agric. Chem.* **1953**, *36*, 445–457. [CrossRef]
22. Box, J.D. Investigation of the Folin-Ciocalteau Phenol Reagent for the Determination of Polyphenolic Substances in Natural Waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
23. von Mersi, W.; Schinner, F. An Improved and Accurate Method for Determining the Dehydrogenase Activity of Soils with Iodonitrotetrazolium Chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
24. Beck, T. Die Messung Der Katalaseaktivität von Böden. *Z. Für Pflanzenernährung Und Bodenkd.* **1971**, *130*, 68–81. [CrossRef]
25. Dick, R.P.; Breakwell, D.P.; Turco, R.F. Soil Enzyme Activities and Biodiversity Measurements as Integrative Microbiological Indicators. In *Methods for Assessing Soil Quality*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 1997; pp. 247–271. ISBN 978-0-89118-944-2.
26. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An Extraction Method for Measuring Soil Microbial Biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
27. Walkley, A.; Black, I.A. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]

28. Bano, R.; Roy, S. Extraction of Soil Microarthropods: A Low Cost Berlese-Tullgren Funnels Extractor. *Int. J. Fauna Biol. Stud.* **2016**, *2*, 14–17.
29. Angelini, P.; Fenoglio, S.; Isaia, M.; Jacomini, C.; Migliorini, M.; Morisi, A. *Biomonitoraggio*. In *Tecniche di Biomonitoraggio del Suolo*; cedam: Torino, Italy, 2002.
30. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. [CrossRef]
31. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant Activity of Some Algerian Medicinal Plants Extracts Containing Phenolic Compounds. *Food Chem.* **2006**, *97*, 654–660. [CrossRef]
32. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free. Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
33. Barreca, D.; Bellocco, E.; Caristi, C.; Leuzzi, U.; Gattuso, G. Flavonoid Composition and Antioxidant Activity of Juices from Chinotto (Citrus × Myrtifolia Raf.) Fruits at Different Ripening Stages. *J. Agric. Food Chem.* **2010**, *58*, 3031–3036. [CrossRef]
34. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. [CrossRef]
35. Davies, S.H.R.; Masten, S.J. Spectrophotometric Method for Ascorbic Acid Using Dichlorophenolindophenol: Elimination of the Interference Due to Iron. *Anal. Chim. Acta* **1991**, *248*, 225–227. [CrossRef]
36. Kruger, N.J. The Bradford Method For Protein Quantitation. In *The Protein Protocols Handbook*; Walker, J.M., Ed.; Humana Press: Totowa, NJ, USA, 2009; pp. 17–24. ISBN 978-1-59745-198-7.
37. Hedge, J.E.; Hofreiter, B.T.; Whistler, R.L. *Carbohydrate Chemistry*; Academic Press: New York, NY, USA, 1962; Volume 17, pp. 71–80.
38. Țopa, D.-C.; Căpșună, S.; Calistru, A.-E.; Ailincăi, C. Sustainable Practices for Enhancing Soil Health and Crop Quality in Modern Agriculture: A Review. *Agriculture* **2025**, *15*, 998. [CrossRef]
39. Lazcano, C.; Domínguez, J. The Use of Vermicompost in Sustainable Agriculture: Impact on Plant Growth and Soil Fertility. *Soil Nutr.* **2011**, *10*, 187.
40. Liu, X.; Yang, Y.; Gao, B.; Li, Y.; Wan, Y. Environmentally Friendly Slow-Release Urea Fertilizers Based on Waste Frying Oil for Sustained Nutrient Release. *ACS Sustain. Chem. Eng.* **2017**, *5*, 6036–6045. [CrossRef]
41. Bernal, M.P.; Alburquerque, J.A.; Moral, R. Composting of Animal Manures and Chemical Criteria for Compost Maturity Assessment. A Review. *Bioresour. Technol.* **2009**, *100*, 5444–5453. [CrossRef]
42. Liu, H.; Du, X.; Li, Y.; Han, X.; Li, B.; Zhang, X.; Li, Q.; Liang, W. Organic Substitutions Improve Soil Quality and Maize Yield through Increasing Soil Microbial Diversity. *J. Clean. Prod.* **2022**, *347*, 131323. [CrossRef]
43. Lori, M.; Symnaczik, S.; Mäder, P.; Deyn, G.D.; Gattinger, A. Organic Farming Enhances Soil Microbial Abundance and Activity—A Meta-Analysis and Meta-Regression. *PLoS ONE* **2017**, *12*, e0180442. [CrossRef] [PubMed]
44. Sritongon, N.; Sarin, P.; Theerakulpisut, P.; Riddech, N. The Effect of Salinity on Soil Chemical Characteristics, Enzyme Activity and Bacterial Community Composition in Rice Rhizospheres in Northeastern Thailand. *Sci. Rep.* **2022**, *12*, 20360. [CrossRef] [PubMed]
45. Sharma, S.; Vashisht, B.B.; Singh, P.; Singh, Y. Changes in Soil Aggregate-Associated Organic Carbon, Enzymatic Activity, and Biological Pools under Conservation Agriculture Based Practices in Rice–Wheat System. *Biomass Convers. Biorefinery* **2023**, *13*, 13977–13994. [CrossRef]
46. Song, R.; Zhu, W.Z.; Li, H.; Wang, H. Impact of Wine-Grape Continuous Cropping on Soil Enzyme Activity and the Composition and Function of the Soil Microbial Community in Arid Areas. *Front. Microbiol.* **2024**, *15*, 1348259. [CrossRef]
47. Mulatu, G.; Bayata, A. Vermicompost as Organic Amendment: Effects on Some Soil Physical, Biological Properties and Crops Performance on Acidic Soil: A Review. *Finite Elem. Method* **2024**, *10*, 66–73. [CrossRef]
48. Rodríguez-Pajares, C.; Muñoz-Adalia, E.J.; Fernández-Fernández, M. Microarthropods Communities as Indicators of Soil Quality in a Mediterranean Periurban Forest Using the QBS-Ar Index. *For. Syst.* **2025**, *34*, 20906. [CrossRef]
49. Churchland, C.; Grayston, S.J. Specificity of Plant-Microbe Interactions in the Tree Mycorrhizosphere Biome and Consequences for Soil C Cycling. *Front. Microbiol.* **2014**, *5*, 261. [CrossRef]
50. Kim, Y.-N.; Lee, J.-H.; Seo, H.-R.; Kim, J.-W.; Cho, Y.-S.; Lee, D.; Kim, B.-H.; Yoon, J.-H.; Choe, H.; Lee, Y.B.; et al. Co-Responses of Soil Organic Carbon Pool and Biogeochemistry to Different Long-Term Fertilization Practices in Paddy Fields. *Plants* **2022**, *11*, 3195. [CrossRef]
51. Reganold, J.P.; Wachter, J.M. Organic Agriculture in the Twenty-First Century. *Nat. Plants* **2016**, *2*, 15221. [CrossRef] [PubMed]
52. Coleman, D.C.; Geisen, S.; Wall, D.H. Chapter 5-Soil Fauna: Occurrence, Biodiversity, and Roles in Ecosystem Function. In *Soil Microbiology, Ecology and Biochemistry*, 5th ed.; Paul, E.A., Frey, S.D., Eds.; Elsevier: Amsterdam, The Netherlands, 2024; pp. 131–159. ISBN 978-0-12-822941-5.

53. Muscolo, A.; Marra, F.; Canino, F.; Maffia, A.; Mallamaci, C.; Russo, M. Growth, Nutritional Quality and Antioxidant Capacity of Lettuce Grown on Two Different Soils with S-Based Fertilizer, Organic and Chemical Fertilizers. *Sci. Hortic.* **2022**, *305*, 111421. [CrossRef]
54. Liu, Y.; Lan, X.; Hou, H.; Ji, J.; Liu, X.; Lv, Z. Multifaceted Ability of Organic Fertilizers to Improve Crop Productivity and Abiotic Stress Tolerance: Review and Perspectives. *Agronomy* **2024**, *14*, 1141. [CrossRef]
55. Laing, W.A.; Martínez-Sánchez, M.; Wright, M.A.; Bulley, S.M.; Brewster, D.; Dare, A.P.; Rassam, M.; Wang, D.; Storey, R.; Macknight, R.C.; et al. An Upstream Open Reading Frame Is Essential for Feedback Regulation of Ascorbate Biosynthesis in Arabidopsis. *Plant Cell* **2015**, *27*, 772–786. [CrossRef]
56. Howe, J.A.; McDonald, M.D.; Burke, J.; Robertson, I.; Coker, H.; Gentry, T.J.; Lewis, K.L. Influence of Fertilizer and Manure Inputs on Soil Health: A Review. *Soil Secur.* **2024**, *16*, 100155. [CrossRef]
57. Ollio, I.; Santás-Miguel, V.; Gómez, D.S.; Lloret, E.; Sánchez-Navarro, V.; Martínez-Martínez, S.; Egea-Gilabert, C.; Fernández, J.A.; Calviño, D.F.; Zornoza, R. Effect of Biofertilizers on Broccoli Yield and Soil Quality Indicators. *Horticulturae* **2023**, *10*, 42. [CrossRef]
58. Singh, S.; Singh, R.; Singh, K.; Katoch, K.; Zaeen, A.A.; Birhan, D.A.; Singh, A.; Sandhu, H.S.; Singh, H.; Sahrma, L.K. Smart Fertilizer Technologies: An Environmental Impact Assessment for Sustainable Agriculture. *Smart Agric. Technol.* **2024**, *8*, 100504. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Chapter 5 – Waste-Derived Fertilizers Enhance Soil Functionality: AMulti-Indicator Assessment in Mediterranean Agroecosystems

Mariateresa Oliva[1], Federica Marra[1], Ludovica Santoro[1], Angela Maffia[1], Santo Battaglia[1], Emilio Attinà[1], Carmelo Mallamaci[1] and Adele Muscolo[1*].

[1] Department of AGRARIA, "Mediterranea" University, 89122 Reggio Calabria, Italy;

[*]Correspondence: amuscolo@unirc.it

Enviroments

https://doi.org/10.3390/environments13060315

Received: 30 April 2026

Revised: 25 May 2026

Accepted: 1 June 2026

Published: 4 June 2026

environments

MDPI

Article

Waste-Derived Fertilizers Enhance Soil Functionality: A Multi-Indicator Assessment in Mediterranean Agroecosystems

Mariateresa Oliva, Federica Marra, Ludovica Santoro, Angela Maffia, Santo Battaglia, Emilio Attinà, Carmelo Mallamaci and Adele Muscolo *

Department of AGRARIA, "Mediterranea" University, 89122 Reggio Calabria, Italy; mariateresa.oliva@unirc.it (M.O.); federica.marra@unirc.it (F.M.); ludovica.santoro@unirc.it (L.S.); angela.maffia@unirc.it (A.M.); santo.battaglia@unirc.it (S.B.); eattina@unirc.it (E.A.); carmelo.mallamaci@unirc.it (C.M.)

* Correspondence: amuscolo@unirc.it

Abstract

Soil degradation and organic matter depletion threaten the sustainability of Mediterranean agroecosystems, highlighting the need for effective and sustainable soil restoration strategies. This study evaluated the short-term effects of composts and vermicomposts derived from chestnut sawdust and food waste on soil functionality and broccoli quality under field conditions using a multi-indicator assessment framework. Six fertilization treatments, including composts, vermicomposts, horse manure, mineral NPK fertilization, and an unfertilized control, were tested in broccoli-cultivated plots. Organic amendments significantly improved soil chemical, biochemical, and biological properties compared with mineral fertilization and the unfertilized control. Vermicompost 10/90 (10% sawdust:90% wet waste) produced the strongest effects, increasing soil organic carbon and organic matter by about 85%, cation exchange capacity by 45%, and dehydrogenase activity by 83% compared with the unfertilized control. Compost and vermicompost treatments also enhanced microbial biomass carbon, enzymatic activities, and QBS-ar values, indicating improved soil biological quality and microarthropod diversity. Broccoli quality was significantly influenced by fertilization regime. Vermicompost 10/90 increased vitamin C by 154%, vitamin E by 54%, total proteins by 18%, and total carbohydrates by 17% compared with the unfertilized control. Organic amendments also enhanced total phenolics, flavonoids, and antioxidant activity relative to NPK and control treatments. Principal component and correlation analyses revealed strong positive relationships among organic matter accumulation, microbial activity, enzymatic processes, soil biodiversity, and crop nutritional quality. Overall, the integrated multi-indicator approach demonstrated that waste-derived organic amendments improve soil functionality and crop quality simultaneously, supporting their use as sustainable tools for circular and resilient Mediterranean agricultural systems.

Keywords: circular economy; mediterranean agroecosystems; multi-indicator assessment; soil biodiversity; soil health indicators; waste-derived fertilizers

Check for updates

Academic Editors: Elias Afif Khouri and Rubén Forján Castro

Received: 30 April 2026
Revised: 25 May 2026
Accepted: 1 June 2026
Published: 4 June 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Healthy soils are essential for agricultural productivity, ecosystem stability, and the regulation of biogeochemical cycles under changing climatic conditions [1]. However, soil quality is increasingly threatened by intensive agricultural practices, excessive mineral fertilization, and the progressive depletion of organic matter, which together represent

major constraints to the sustainability of cropping systems worldwide [2]. Soil degradation is characterized by declines in organic carbon, microbial biomass, nutrient cycling efficiency, and soil faunal diversity, ultimately impairing key soil functions [3]. These processes are particularly severe in Mediterranean regions, where hydroclimatic variability, water scarcity, and erosion accelerate degradation and reduce the capacity of soils to sustain ecosystem services [4]. Consequently, sustainable soil management strategies capable of restoring organic matter and biological functioning are urgently required.

Organic amendments have received increasing attention as sustainable tools for improving soil fertility and functionality because they supply both labile and stabilized organic carbon, stimulate microbial activity, and enhance soil structure and water retention [5,6]. In this context, the recycling of organic wastes through composting and vermicomposting represents a promising approach within circular economy systems, allowing the recovery of nutrients while reducing environmental impacts. Vermicomposts, in particular, are characterized by high humic content, active microbial communities, and readily available organic compounds, which can strongly influence soil biochemical and biological processes [7]. Despite these advantages, field-based evidence integrating multiple soil quality indicators to assess short-term responses to organic amendments remains limited, especially in Mediterranean environments characterized by low organic matter content and reduced functional resilience [8]. Because soil quality cannot be directly measured, its evaluation relies on a suite of complementary indicators describing physical, chemical, biochemical, microbiological, and faunal components [9]. Among these, biological and biochemical parameters—such as enzymatic activities, microbial biomass carbon (MBC), cation exchange capacity (CEC), and soil biodiversity indices (e.g., QBS-ar)—are particularly sensitive in detecting short-term changes induced by management practices [10,11]. Nevertheless, the relationships linking organic amendment composition, microbial stimulation, enzymatic activity, soil biodiversity, and crop nutritional quality are still insufficiently explored under real agricultural conditions.

Therefore, a multi-indicator framework is essential to capture the complexity of soil responses and to identify early changes in soil functionality following organic amendment application. The novelty of the present study lies in the simultaneous integration of chemical, biochemical, microbiological, faunal (QBS-ar), and broccoli nutritional indicators in a field-based experiment under Mediterranean conditions. We hypothesize that organic amendments will produce measurable short-term improvements in soil biological and biochemical indicators, enhancing overall soil functionality compared to unamended and mineral-fertilized soils.

The objectives of this study are to:

I. quantify short-term changes in soil physical, chemical, biochemical, and microbiological properties following organic amendment application;
II. identify the most sensitive indicators for detecting early soil quality changes under Mediterranean conditions;
III. evaluate the effectiveness of compost and vermicompost derived from organic wastes in improving soil functionality.

This study provides a field-based, integrative assessment of soil responses to organic amendments under Mediterranean conditions, contributing to the understanding of how waste-derived fertilizers can enhance soil functionality and support sustainable soil management.

2. Materials and Methods

2.1. Experimental Site, Treatments and Soil Sampling

The field experiment was conducted at Orfei Farm, located in the municipality of Motta San Giovanni (southern Italy), at an altitude of 450 m a.s.l. The area is characterized

by a semi-arid Mediterranean climate, with pronounced seasonal variability and soils typical of environments prone to degradation processes. According to the FAO classification system [12], the experimental soil was identified as a sandy clay loam with a particle size distribution of approximately 65% sand, 23% silt, and 12% clay. Experimental plots (18 m^2 each) were arranged in a randomized block design with three replications per treatment. A total of six fertilization treatments were tested, along with an unfertilized control. The treatments included: Compost with a 50/50 chestnut sawdust to wet waste ratio (C50/50); Compost with a 10/90 chestnut sawdust to wet waste ratio (C10/90); Vermicompost with a 50/50 chestnut sawdust to wet waste ratio (V50/50); Vermicompost with a 10/90 chestnut sawdust to wet waste ratio (V10/90); Horse manure (HM); Synthetic NPK fertilizer (20:10:10). In this study, wet waste refers to the biodegradable organic fraction used as the moist, readily degradable component of the composting and vermicomposting mixtures, mainly consisting of food and vegetable residues. The compost and vermicompost treatments were applied at a rate of 3100 kg ha^{-1}, horse manure at 4300 kg ha^{-1}, and the chemical NPK fertilizer at 1700 kg ha^{-1}. The reference crop was Calabrian broccoli (*Brassica oleracea* var. italica), which served as the biological model for evaluating the effects of organic and inorganic fertilization on crop quality. Seedlings were transplanted in open-field conditions using a spacing of 70 cm × 50 cm, corresponding to approximately 28,500 plants ha^{-1}. The crop cycle was conducted from October to February under Mediterranean open-field conditions, with mean air temperatures ranging from 10 to 18 °C, maximum temperatures occasionally reaching 22–24 °C during autumn periods, and cumulative rainfall during the experimental period of approximately 350–450 mm, mainly concentrated between November and January. During the experimental period, all plots were managed uniformly. Irrigation was supplied when required according to crop water demand and seasonal rainfall, weeds were controlled manually/mechanically, and plant protection measures were applied only when necessary following local agronomic practice. No additional fertilization was applied after the initial treatment application.

Soil sampling was carried out at two key stages of the experimental cycle:

(i) T_0—prior to the application of any treatment (baseline conditions);
(ii) T_1—at the end of the experiment, during broccoli harvest;

At each sampling time, five broccoli plants and five composite soil samples were collected from each treatment plot. For the QBS-ar analysis, five additional soil samples were collected per treatment to ensure sufficient replication for faunal community assessment. Soil samples were taken from the 0–20 cm soil layer, after removing surface residues. Soil samples intended for physicochemical analyses were air-dried at room temperature, gently crushed, homogenized, and sieved through a 2 mm mesh before analysis. Fresh soil subsamples intended for microbiological and enzymatic determinations were immediately placed in sterile polyethylene bags, transported to the laboratory in cooled containers, and stored at 4 °C until analysis. These analyses were performed within 48 h after sampling to minimize changes in microbial and enzymatic activity.

For QBS-ar analysis, five additional undisturbed soil samples were collected from each treatment to ensure sufficient replication for faunal community assessment. These samples were kept fresh, transported to the laboratory under cool conditions, and processed immediately for microarthropod extraction.

At harvest, five broccoli plants per treatment plot were randomly selected. Edible portions were separated, washed with distilled water, gently dried with absorbent paper, homogenized, and stored at −20 °C until nutritional and nutraceutical analyses. For dry matter determination, aliquots of fresh plant material were oven-dried at 65 °C until constant weight.

2.2. Chemical, Biochemical, and Microbiological Analyses of Soil

Soil characterization was performed using an integrated set of physical, chemical, biochemical, and microbiological determinations, identified as fundamental indicators of soil quality according to a multi-indicator framework. Soil water content (WC) was determined gravimetrically by oven-drying samples at 105 °C [13], until constant weight using a ventilated laboratory oven (Memmert UF55, Memmert GmbH, Schwabach, Germany). Soil texture was analyzed using the Bouyoucos hydrometer method [14]. Electrical conductivity (EC) was measured in a 1:5 soil-to-water suspension after 1 h of controlled agitation (15 rpm) using a conductivity meter (HI-993310, Hanna Instruments, Villafranca Padovana, Italy), and soil pH was determined in distilled water (1:2.5 w/v) using a glass electrode pH meter equipped with a glass electrode (Crison Basic 20+, Crison Instruments, Alella, Spain). Organic carbon (OC) and total nitrogen (TN) contents were quantified using a LECO CN628 elemental analyzer. Organic matter (OM) was estimated by multiplying OC by a conversion factor of 1.72, and the C/N ratio was subsequently calculated. The cation exchange capacity (CEC), considered a functional indicator of soil fertility, was determined according to the method of Mehlich et al. [15]. Water-extractable phenolic compounds, representing the labile organic fraction, were quantified using the Folin–Ciocalteu method [16] and measured spectrophotometrically using a UV–Vis spectrophotometer (UV-1800, Shimadzu Corporation, Kyoto, Japan), with results expressed as gallic acid equivalents (GAE). Enzymatic activities were evaluated as proxies of soil biochemical functioning: dehydrogenase activity (DHA) was determined according to [17] as an indicator of overall microbial metabolic activity; catalase activity (CAT) was measured volumetrically by quantifying oxygen evolution following incubation with hydrogen peroxide [18]; and fluorescein diacetate hydrolysis (FDA), representing overall hydrolytic enzymatic potential, was determined following Dick et al. [19]. Absorbance measurements for enzymatic assays were performed using the UV–Vis spectrophotometer described above. Microbial biomass carbon (MBC) was assessed using the chloroform fumigation–extraction method [20] with 20 g oven-dry equivalent subsamples. Fumigated and non-fumigated extracts were analyzed for soluble organic carbon by the Walkley–Black procedure [21], applying a correction factor of 0.38 to estimate MBC. Data are the mean of 5 replications.

2.3. Soil Fauna Analysis

The structure of the soil microarthropod community used as an integrative biological indicator of soil quality was assessed following Bano and Roy [22] and Angelini et al. [23]. For each sample, 1 kg of fresh soil was placed on a 2 mm mesh screen in a Berlese–Tüllgren extraction apparatus above a funnel containing an alcohol:glycerol preservative solution (2:1). Arthropod migration was induced by a 40–60 W incandescent lamp positioned approximately 20 cm above the soil surface. Extraction was maintained for ten days, a duration selected to allow gradual drying of the soil sample and complete migration of microarthropods from the soil matrix into the preservative solution.

Extracted organisms were observed under a stereomicroscope at 20–40× magnification and classified into Biological Forms according to their morphological adaptation to the soil environment. The assignment of each organism to a Biological Form was based on eco-morphological traits such as pigmentation, eye reduction, appendage development, body shape, and degree of adaptation to edaphic life, following the QBS-ar reference procedure [23]. Each Biological Form was assigned an eco-morphological index (EMI), with higher EMI values corresponding to greater adaptation to soil habitats. When multiple Biological Forms belonging to the same taxonomic group were present, the highest EMI value was used, as recommended by Angelini et al. [23].

To reduce potential subjectivity in the assignment of Biological Forms, all samples were examined using the same identification criteria and reference tables, and doubtful

specimens were re-checked before final classification. The QBS-ar index was calculated as the sum of all EMI scores recorded for each sample.

2.4. Nutritional and Nutraceutical Analyses of Broccoli

Broccoli samples were analyzed for water content (WC), dry weight (DW), and a suite of nutritional and nutraceutical quality parameters. WC was determined as the percentage of mass loss following oven-drying at 65 °C until constant weight, while DW was expressed as the residual mass after dehydration. Total phenolic content was quantified by the Folin–Ciocalteu method [16] following the procedure of Velioglu et al. [24]. Total flavonoids were determined using the aluminium chloride colorimetric assay described by Djeridane et al. [25]. Antioxidant activity was assessed using two complementary assays: $ABTS^+$ radical cation decolorization [26] and DPPH radical scavenging capacity [27]. Total antioxidant capacity (TAC) and vitamin E were determined according to Prieto et al. [28], whereas ascorbic acid (vitamin C) was quantified following Davies and Masten [29]. Total protein content was measured using the Bradford assay [30], and total carbohydrates were quantified using the phenol–sulfuric acid method of Hedge et al. [31]. All spectrophotometric measurements were performed using calibrated instruments and analytical-grade reagents. Calibration curves were prepared using appropriate analytical standards for each assay, and all analyses were carried out in triplicate to ensure analytical reproducibility and quality control. Blank samples and reagent controls were included where appropriate to minimize analytical interference and ensure measurement reliability.

2.5. Statistical Analyses

Data normality and homoscedasticity were verified prior to ANOVA. All experimental data were subjected to one-way analysis of variance (ANOVA). Differences among treatments were evaluated using Tukey's Honestly Significant Difference (HSD) test. Multivariate relationships between soil parameters and broccoli biochemical traits were explored through Principal Component Analysis (PCA). Pearson correlation coefficients were calculated to assess linear associations among variables. Statistical analyses were performed using MATLAB R2024b (The MathWorks Inc., Natick, MA, USA), adopting a significance threshold of $p \leq 0.05$.

3. Results

3.1. Effects of Fertilizers on Soil Quality

Table 1 reports the soil properties measured before treatment application (T_0) and after the completion of the fertilization treatments. Initial soil conditions were characterized by an organic carbon (OC) content of 1.37%, a C/N ratio of 7.21, dehydrogenase activity (DHA) of 15.11 μg TTF g^{-1} h^{-1}, microbial biomass carbon (MBC) of 376.1 μg C g^{-1}, and fluorescein diacetate hydrolase (FDA) activity of 2.15 μg g^{-1}. Following fertilization, soil chemical and biochemical properties improved markedly across treatments. Compost and vermicompost applications significantly increased electrical conductivity (EC), with values reaching up to 732 μS cm^{-1}, while soil pH remained relatively stable, indicating no acidification effects. Among treatments, vermicompost 10/90 produced the greatest increase in organic carbon, reaching 3.28%, corresponding to an increase of over 140% compared to baseline conditions. Compost 50/50 resulted in the highest microbial biomass carbon (495.39 μg C g^{-1}), whereas compost 10/90 showed the strongest stimulation of microbial activity, with dehydrogenase activity increasing by 152% (37.07 μg TTF g^{-1} h^{-1}). Cation exchange capacity (CEC) also increased under organic amendments, rising from 16.1 to 23.1 cmol(+) kg^{-1}, indicating enhanced nutrient retention and improved soil fertility.

Table 1. Chemical and biochemical properties of soils before the application of any treatment T_0 and at the broccoli harvest (six months after the treatments). Six fertilization treatments were tested: NPK = nitrogen–phosphorous–potassium; HM = horse manure; C 50/50 = compost with 50% wood sawdust + 50% wet waste; C 10/90 = compost with 10% wood sawdust + 90% wet waste; VC 50/50 = vermicompost with 50% wood sawdust + 50% wet waste; VC 10/90 = vermicompost with 10% wood sawdust + 90% wet waste along with an unfertilized control CTR (control) = soil without fertilizers.

	T_0	CTR	HM	NPK	C 50/50	C 10/90	VC 50/50	VC 10/90
WC	18.2 ^{d} ± 0.8	19.4 ^{d} ± 0.9	21.3 ^{c} ± 1.1	24.1 ^{b} ± 1.2	25.9 ^{a} ± 1.3	25.1 ^{a} ± 1.2	26.8 ^{a} ± 1.4	26.2 ^{a} ± 1.3
pH	8.5 ^{a} ± 0.2	8.3 ^{a} ± 1.8	8.1 ^{a} ± 2.1	8.3 ^{a} ± 2.4	8.4 ^{a} ± 2.6	8.3 ^{a} ± 1.4	8.2 ^{a} ± 3.2	8.1 ^{a} ± 0.9
EC	307.3 ^{d} ± 12.1	329 ^{d} ± 14.2	304 ^{d} ± 13.8	291 ^{d} ± 12.9	535 ^{c} ± 18.4	732 ^{a} ± 21.3	561 ^{c} ± 19.2	611 ^{b} ± 20.1
OC	1.37 ^{d} ± 0.08	1.77 ^{c} ± 0.09	1.61 ^{c} ± 0.08	2.09 ^{b} ± 0.11	2.91 ^{a} ± 0.14	3.25 ^{a} ± 0.16	3.01 ^{a} ± 0.15	3.28 ^{a} ± 0.16
TN	0.19 ^{b} ± 0.01	0.18 ^{b} ± 0.01	0.21 ^{a} ± 0.02	0.23 ^{a} ± 0.02	0.18 ^{b} ± 0.01	0.21 ^{a} ± 0.02	0.14 ^{c} ± 0.01	0.15 ^{c} ± 0.01
OM	2.36 ^{d} ± 0.12	3.05 ^{c} ± 0.14	2.78 ^{c} ± 0.13	3.60 ^{b} ± 0.16	5.02 ^{a} ± 0.21	5.60 ^{a} ± 0.23	5.19 ^{a} ± 0.22	5.65 ^{a} ± 0.24
C/N	7.21 ^{d} ± 0.34	9.83 ^{c} ± 0.42	7.67 ^{d} ± 0.36	9.09 ^{c} ± 0.41	16.17 ^{b} ± 0.68	15.48 ^{b} ± 0.65	21.50 ^{a} ± 0.89	21.87 ^{a} ± 0.91
WSP	276.1 ^{a} ± 11.8	286 ^{a} ± 12.4	318 ^{a} ± 13.9	310 ^{a} ± 13.6	142 ^{c} ± 7.2	176 ^{b} ± 8.8	289 ^{a} ± 12.6	277 ^{a} ± 12.1
CEC	16.1 ^{b} ± 0.7	15.9 ^{b} ± 0.7	12.5 ^{c} ± 0.6	20.3 ^{a} ± 0.9	21.6 ^{a} ± 0.9	22.8 ^{a} ± 1.0	21.2 ^{a} ± 0.9	23.1 ^{a} ± 1.0
DHA	15.11 ^{d} ± 0.82	19.76 ^{c} ± 1.05	23.12 ^{b} ± 1.21	25.43 ^{b} ± 1.32	33.14 ^{a} ± 1.67	37.07 ^{a} ± 1.84	33.11 ^{a} ± 1.66	36.14 ^{a} ± 1.79
FDA	2.15 ^{b} ± 0.14	4.54 ^{a} ± 0.26	5.63 ^{a} ± 0.31	5.39 ^{a} ± 0.30	4.98 ^{a} ± 0.28	4.89 ^{a} ± 0.27	5.54 ^{a} ± 0.31	5.87 ^{a} ± 0.33
CAT	1.26 ^{e} ± 0.08	3.76 ^{a} ± 0.21	3.17 ^{b} ± 0.18	2.06 ^{c} ± 0.13	1.98 ^{c} ± 0.12	1.96 ^{c} ± 0.12	1.84 ^{c} ± 0.11	1.56 ^{d} ± 0.10
MBC	376.1 ^{b} ± 18.2	434.17 ^{a} ± 20.8	432.47 ^{a} ± 20.7	494.98 ^{a} ± 23.1	495.39 ^{a} ± 23.2	462.98 ^{a} ± 21.9	454.29 ^{a} ± 21.6	424.11 ^{a} ± 20.4

WC = water content (%); pH; EC = electric conductivity (dS/m); OC = organic carbon (%); TN = total nitrogen (%); OM = organic matter (%); C/N = carbon nitrogen ratio; WSP = water-soluble phenols (μg TAEg^{-1}ds); CEC = cation exchange capacity (cmol (+) Kg^{-1} d.s.); DHA = dehydrogenase (μg TTFg^{-1} h^{-1} d.s.); FDA = fluorescein diacetate hydrolase (μg fluoresceing^{-1} d.s.); CAT = catalase (O_2%/3 min/g); MBC = microbial biomass carbon (μgC g^{-1} f.s.). Data are the means of five replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

Principal component analysis (PCA; Figure 1) revealed a clear separation of soil parameters according to fertilization treatments. Control (CTR), NPK, and horse manure (HM) were located in the upper left quadrant and were positively associated with total nitrogen (TN) and catalase activity (CAT). Vermicompost treatments (VC 50/50 and VC 10/90) were primarily distributed in the lower right quadrant, showing strong associations with pH, C/N ratio, electrical conductivity (EC), and cation exchange capacity (CEC). Compost treatments (C50/50 and C10/90) clustered in the upper region of the biplot and were closely related to dehydrogenase activity (DHA), microbial biomass carbon (MBC), organic matter (OM), and fluorescein diacetate hydrolase (FDA). The T_0 samples were positioned in the lower left quadrant, with negative scores on both principal components, reflecting generally low values across all measured soil parameters.

Pearson correlation analysis (Figure 2) revealed significant relationships among the chemical and biological soil parameters. Very strong positive correlations (r > 0.90) were observed between organic carbon (OC) and organic matter (OM) (r = 0.987), OC and dehydrogenase activity (DHA) (r = 0.968), and OM and DHA (r = 0.976). Strong positive correlations were also found between electrical conductivity (EC) and OM (r = 0.935), EC and OC (r = 0.913), and EC and DHA (r = 0.903). Water content (WC) was positively correlated with OC (r = 0.847), OM (r = 0.870), DHA (r = 0.914), and cation exchange capacity (CEC) (r = 0.760). CEC showed strong positive correlations with OM (r = 0.901), OC (r = 0.884), and DHA (r = 0.832). In contrast, total nitrogen (TN) was negatively correlated with most soil parameters, particularly with the C/N ratio (r = −0.758), OM (r = −0.475), and EC (r = −0.456). Catalase activity (CAT) also showed negative correlations with CEC (r = −0.590), OM (r = −0.395), and OC (r = −0.364). Soil pH exhibited weak or negligible correlations with most of the analysed parameters.

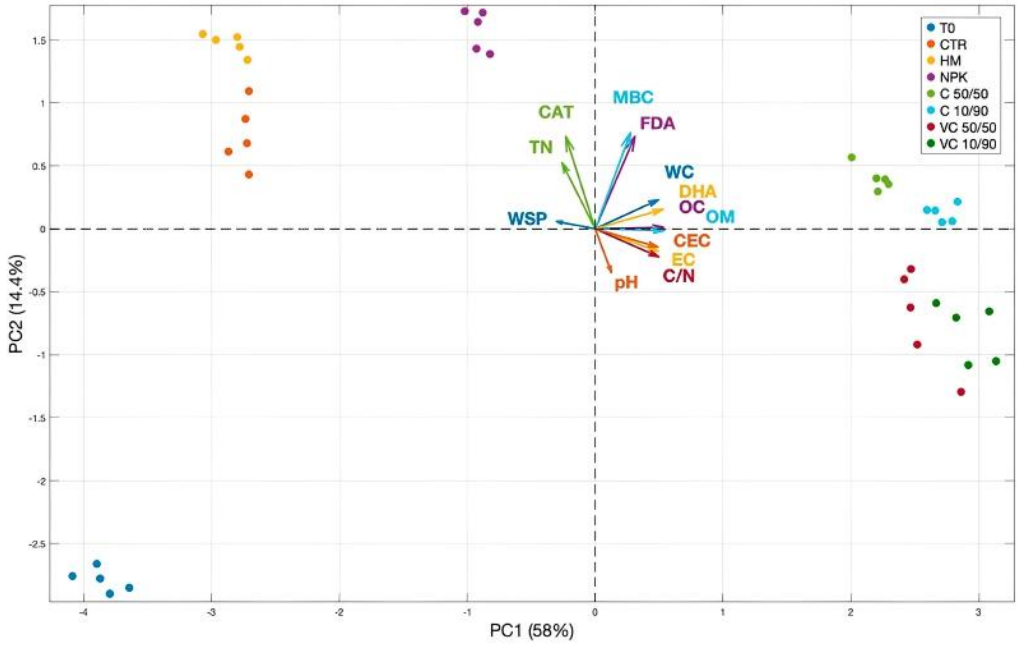


Figure 1. Principal component analysis (PCA) analysis of the chemical and physical properties of the different treatments. T_0 = before the application of any treatment; CTR (control) = soil without fertilize; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C 50/50 = compost with 50% wood sawdust + 50% wet waste; C 10/90 = compost with 10% wood sawdust + 90% wet waste; VC 50/50 = vermicompost with 50% wood sawdust + 50% wet waste and VC 10/90 = vermicompost with 10% wood sawdust + 90% wet waste. WC = water content; pH; EC = electric conductivity; OC = organic carbon; TN = total nitrogen; OM = organic matter; C/N = carbon nitrogen ratio; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase; FDA = fluorescein diacetate hydrolase; CAT = catalase; MBC = microbial biomass carbon.

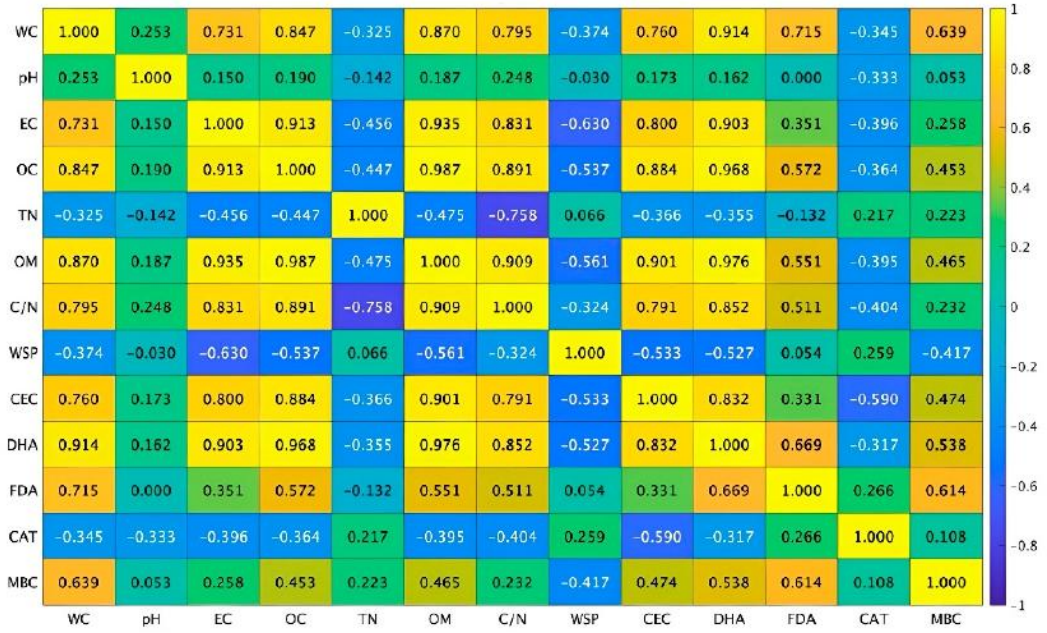


Figure 2. Pearson correlation matrix illustrating the relationships among biochemical, chemical, and physical soil parameters. Correlation coefficients range from −1 (strong negative correlation, in dark blue) to +1 (strong positive correlation, in yellow), as shown by the color scale on the right. WC = water content; pH = potential of hydrogen; EC = electrical conductivity; OC = organic carbon; TN = total nitrogen; OM = organic matter; C/N = carbon–nitrogen ratio; WSP = water-soluble phenols; CEC = cation exchange capacity; DHA = dehydrogenase activity; FDA = fluorescein diacetate hydrolase; CAT = catalase; MBC = microbial biomass carbon.

3.2. QBS-ar Index

The QBS-ar index showed significant variation among fertilization treatments (Figure 3). Organic waste-derived amendments markedly increased QBS-ar values compared to both the unfertilized control (CTR) and the NPK treatment. Among the treatments, vermicompost 10/90 exhibited the highest QBS-ar values, indicating the strongest enhancement of soil biological quality, followed by both compost treatments (C50/50 and C10/90).

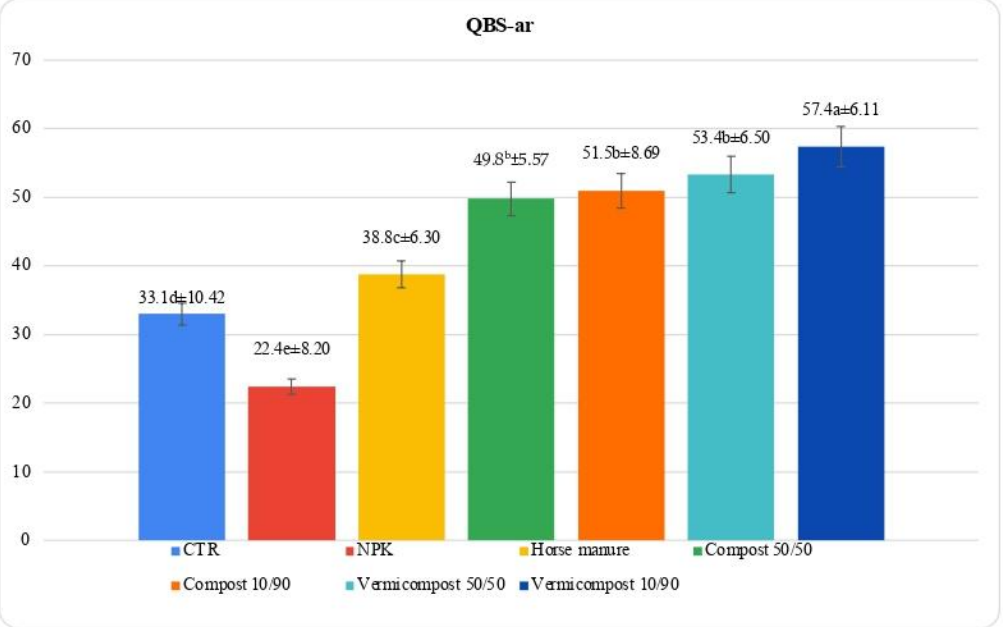


Figure 3. The QBS-ar index. CTR (control) = unfertilized soil; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C 50/50 = compost with 50% wood sawdust + 50% wet waste; C 10/90 = compost with 10% wood sawdust + 90% wet waste; VC 50/50 = vermicompost with 50% wood sawdust + 50% wet waste and VC 10/90 = vermicompost with10% wood sawdust + 90% wet waste. Data are the means of five replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

In contrast, soils treated with NPK showed the lowest QBS-ar scores, even lower than the unfertilized control, suggesting a reduced abundance and ecological adaptation of soil microarthropod communities under mineral fertilization. Horse manure showed intermediate values, indicating a moderate improvement in soil biological quality compared to synthetic fertilization but lower effectiveness than compost and vermicompost.

Overall, the QBS-ar index clearly discriminated among fertilization strategies, highlighting the positive effect of organic waste-based amendments on soil faunal biodiversity and biological quality.

3.3. Broccoli Quality

Broccoli grown in soils amended with organic waste-based fertilizers exhibited enhanced nutritional and nutraceutical profiles compared to T_0, the unfertilized control (CTR), and NPK treatments (Table 2). The highest total phenolic contents were recorded in plants treated with compost 10/90 (58.62 mg GAE g^{-1} dw) and vermicompost 10/90 (58.19 mg GAE g^{-1} dw). Total flavonoid concentrations were significantly higher in all organic amendment treatments compared to T_0, CTR, and horse manure (HM), with the greatest values observed under C10/90 and VC10/90. Antioxidant activity, assessed by DPPH, $ABTS^+$, and total antioxidant capacity (TAC) assays, was highest in broccoli treated with vermicomposts, whereas the lowest values were observed in the unfertilized control. Vermicompost 10/90 also resulted in the greatest increase in vitamin content, with vitamin

C reaching 54.38 mg ascorbic acid 100 g^{-1} dw and vitamin E 8.18 mg α-tocopherol 100 g^{-1} dw, compared to 21.38 and 5.31 mg dw in CTR, and 39.62 and 5.90 mg dw in NPK, respectively. Organic amendments further enhanced primary metabolites, with increased total protein and carbohydrate contents compared to CTR, NPK, and HM. Among all treatments, vermicompost 10/90 showed the strongest effect, yielding the highest concentrations of total protein (93.21 mg BSA g^{-1} dw) and total carbohydrates (139.08 mg glucose g^{-1} dw).

Table 2. Analysis of broccoli grown in soil differently treated: NPK = nitrogen–phosphorous–potassium; HM = horse manure; C 50/50 = compost with 50% wood sawdust + 50% wet waste; C 10/90 = compost with 10% wood sawdust + 90% wet waste; VC 50/50 = vermicompost with 50% wood sawdust + 50% wet waste; VC 10/90 = vermicompost with 10% wood sawdust + 90% wet waste along with an unfertilized control CTR (control) = soil without fertilizers.

	CTR	NPK	HM	C 50/50	C 10/90	VC 50/50	VC 10/90
WC	86.4 ^{a} ± 0.3	84.7 ^{a} ± 0.4	85.3 ^{a} ± 0.2	85.2 ^{a} ± 0.3	85.6 ^{a} ± 0.3	86.0 ^{a} ± 0.2	85.6 ^{a} ± 0.2
DW	13.6 ^{a} ± 0.2	15.3 ^{a} ± 0.3	14.7 ^{a} ± 0.2	14.8 ^{a} ± 0.2	14.4 ^{a} ± 0.2	14.0 ^{a} ± 0.3	14.4 ^{a} ± 0.3
TP	53.12 ^{c} ± 0.48	56.72 ^{a} ± 0.52	56.10 ^{b} ± 0.45	56.81 ^{a} ± 0.51	58.62 ^{a} ± 0.46	56.43 ^{b} ± 0.47	58.19 ^{a} ± 0.44
TF	4.97 ^{d} ± 0.08	5.29 ^{c} ± 0.09	5.98 ^{b} ± 0.07	6.33 ^{ab} ± 0.08	6.41 ^{a} ± 0.06	6.32 ^{ab} ± 0.07	6.35 ^{a} ± 0.06
DPPH	22.61 ^{c} ± 0.14	23.35 ^{b} ± 0.12	23.42 ^{b} ± 0.11	23.57 ^{b} ± 0.14	23.71 ^{b} ± 0.11	24.10 ^{a} ± 0.09	24.02 ^{a} ± 0.10
$ABTS^{+}$	33.47 ^{b} ± 0.18	32.79 ^{b} ± 0.17	33.39 ^{b} ± 0.09	33.02 ^{b} ± 0.16	33.4 ^{b} ± 0.09	33.68 ^{a} ± 0.14	33.88 ^{a} ± 0.21
TAC	1.49 ^{c} ± 0.03	1.52 ^{c} ± 0.03	1.68 ^{b} ± 0.04	1.75 ^{b} ± 0.03	1.72 ^{b} ± 0.03	1.84 ^{a} ± 0.02	1.82 ^{a} ± 0.02
Vit C	21.38 ^{g} ± 0.42	39.62 ^{f} ± 0.55	41.05 ^{e} ± 0.48	43.74 ^{d} ± 0.46	47.53 ^{c} ± 0.49	52.58 ^{b} ± 0.51	54.38 ^{a} ± 0.52
Vit E	5.31 ^{d} ± 0.09	5.90 ^{c} ± 0.10	6.09 ^{c} ± 0.09	7.26 ^{b} ± 0.08	7.36 ^{b} ± 0.07	8.21 ^{a} ± 0.09	8.18 ^{a} ± 0.08
Tot Pro	79.29 ^{e} ± 0.85	80.46 ^{e} ± 0.81	83.21 ^{d} ± 0.77	89.10 ^{c} ± 0.73	90.12 ^{b} ± 0.30	91.34 ^{a} ± 0.91	93.21 ^{a} ± 0.97
Tot Carb	119.15 ^{d} ± 1.02	121.09 ^{d} ± 0.95	121.52 ^{d} ± 0.91	131.36 ^{c} ± 0.88	136.39 ^{b} ± 0.86	135.06 ^{b} ± 0.84	139.08 ^{a} ± 0.81

WC = water content (%); DW = dry weight (%); TP = total phenols (mg GAE g^{-1} dw); TF = total flavonoids (mg QE g^{-1}); DPPH = 2,2-difenil-1-picrilidrazile (inhibition %); $ABTS^{+}$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato (μM Trolox g^{-1} dw); TAC = total antioxidant capacity (mg α-tocopherol 100g^{-1} dw); Vit C = Vitamin C (mg ascorbic acid 100g^{-1} dw); Vit E = Vitamin E (mg α-tocopherol 100g^{-1} dw); Total Pro = Tot Protein (mg BSA g^{-1} dw); Tot Carb = Total Carbohydrates (mg Glucose g^{-1} dw). Data are the means of five replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

PCA biplot analysis (Figure 4) showed a clear separation of treatments based on broccoli nutritional and nutraceutical traits. The unfertilized control (CTR), NPK, and horse manure (HM) were located in the left quadrant and showed no strong association with the measured quality parameters. CTR was positioned in the upper left quadrant, whereas NPK and HM were located in the lower left quadrant, all characterized by negative scores along PC1. In contrast, vermicompost treatments (VC50/50 and VC10/90) were distributed in the upper right quadrant and were positively associated with total carbohydrates, total proteins, total antioxidant capacity (TAC), $ABTS^{+}$ activity, and vitamin E, and to a lesser extent with total flavonoids (TF). Compost treatments (C50/50 and C10/90) were mainly located in the lower right quadrant and were positively associated with total phenols (TP), DPPH activity, vitamin C, and dry weight (DW), with a weaker association with TF. Pearson correlation analysis (Figure 5) further highlighted strong relationships among crop quality parameters. Very strong positive correlations ($r > 0.90$) were observed between vitamin E and total proteins ($r = 0.974$), vitamin E and total carbohydrates ($r = 0.948$), and total proteins and total carbohydrates ($r = 0.967$). Strong positive correlations were also found between TF and total proteins ($r = 0.920$), TF and TAC ($r = 0.906$), and DPPH activity and vitamin C ($r = 0.907$). Antioxidant-related parameters were closely interrelated, with strong correlations between TAC and vitamin E ($r = 0.864$), TAC and vitamin C ($r = 0.805$), vitamin C and vitamin E ($r = 0.893$), and vitamin C and total proteins ($r = 0.872$). Total phenols showed positive correlations with TF ($r = 0.762$), DPPH ($r = 0.711$), vitamin C ($r = 0.819$), and total carbohydrates ($r = 0.722$). $ABTS^{+}$ activity exhibited generally moderate correlations with other variables, with slightly higher associations with vitamin E ($r = 0.393$) and DPPH ($r = 0.232$). Water content (WC) was negatively correlated with dry weight (DW) ($r = -0.670$) and total phenols ($r = -0.476$), and showed weak negative correlations with

most other parameters. Dry weight displayed a moderate positive correlation with total phenols (r = 0.563) and weaker associations with the remaining biochemical traits.

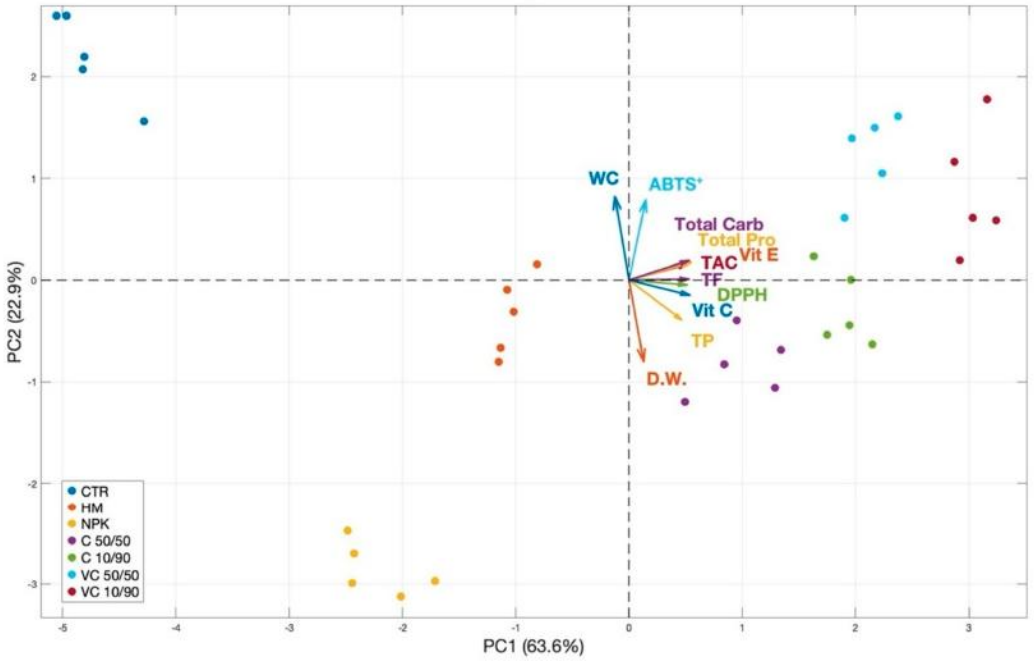


Figure 4. Principal component analysis (PCA) analysis of the chemical and physical properties of the different treatment: CTR (control) = soil without fertilize; HM = horse manure; NPK = nitrogen–phosphorous–potassium; C 50/50 = compost with 50% wood sawdust + 50% wet waste; C 10/90 = compost with 10% wood sawdust + 90% wet waste; VC 50/50 = vermicompost with 50% wood sawdust + 50% wet waste and VC 10/90 = vermicompost with10% wood sawdust + 90% wet waste. WC = water content; D.W. = dry weight; Total Carb = total carbohydrates; Total Pro = total proteins; TP = total phenols; TAC = total antioxidant capacity; $ABTS^{+}$ = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato; DPPH = 2,2-difenil-1-picrilidrazile; Vit C = vitamin C; Vit E = vitamin E; TF = total flavonoids.

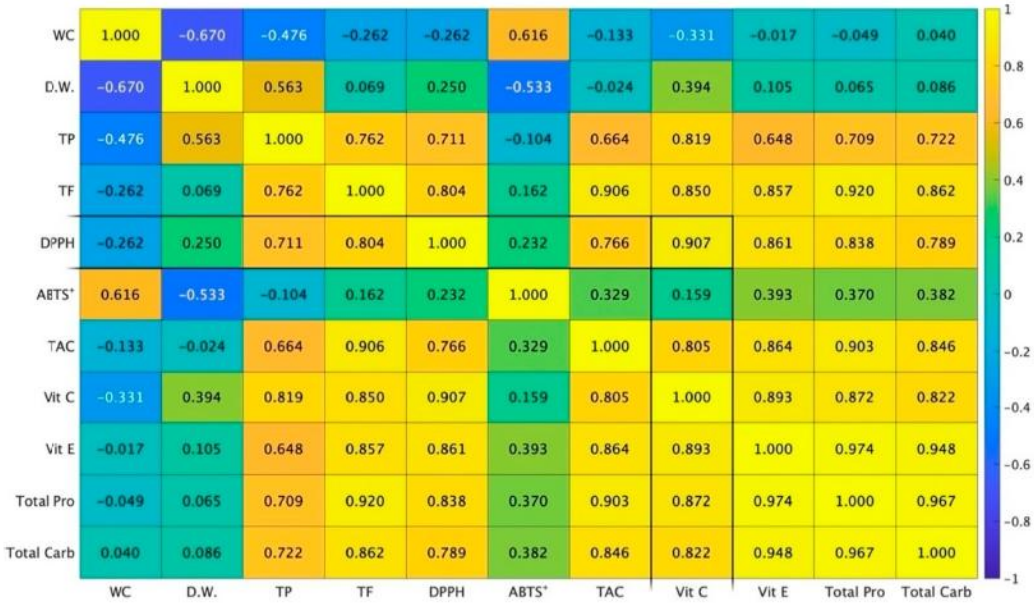


Figure 5. Pearson correlation matrix illustrating the relationships among nutritional and antioxidant parameters of the crop. Correlation coefficients range from −1 (strong negative correlation, in dark blue) to +1 (strong positive correlation, in yellow), as indicated by the color scale on the right. The evaluated variables include: WC = water content; D.W. = dry weight; TP = total phenols; TF = total

flavonoids; DPPH = 2,2-difenil-1-picrilidrazile; ABTS^{+} = 2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato; TAC = total antioxidant capacity; Vit C = vitamin C; Vit E = vitamin E; Total Pro = total proteins; Total Carb = total carbohydrates.

3.4. Multi-Indicator Assessment of Soil Quality

The spider plot (Figure 6) provides an integrated comparison of the relative performance of the main soil quality indicators across treatments. Organic amendments, particularly compost 10/90 and both vermicompost formulations, exhibited the highest normalised values for chemical indicators (CEC, OM, C/N) and biological parameters (DHA, FDA, MBC, QBS-ar), highlighting their strong positive influence on overall soil functionality. In contrast, NPK and the unfertilised control (CTR) showed consistently lower values for most indicators, especially those related to microbial activity and biochemical processes. The multidimensional structure of the plot illustrates that organic waste-based amendments promote a coordinated improvement of both chemical and biological soil properties. Overall, the multi-indicator approach clearly demonstrates the superior effectiveness of organic amendments in enhancing soil quality compared to mineral fertilisation.

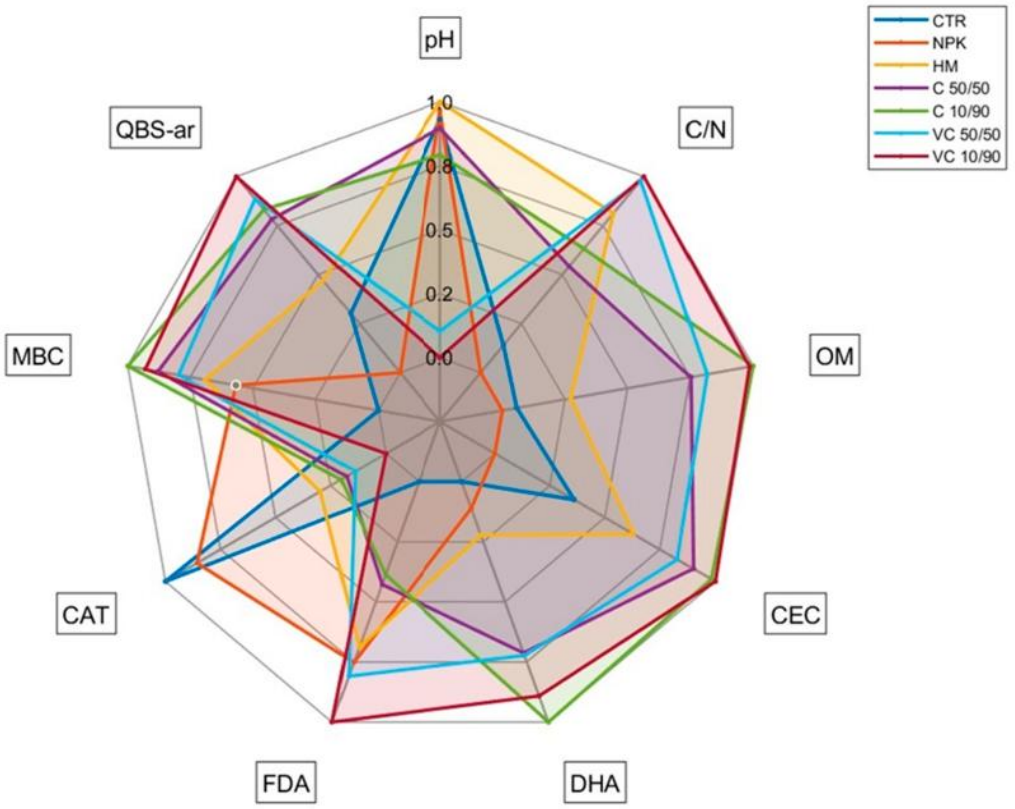


Figure 6. Spider plot illustrating the multi-indicator assessment of soil quality under different fertilization regimes. Normalised values (0–1 scale) are reported for the following soil quality indicators: pH; C/N = carbon-to-nitrogen ratio; OM = organic matter; CEC = cation exchange capacity; DHA = dehydrogenase activity; FDA = fluorescein diacetate hydrolase activity; CAT = catalase activity; MBC = microbial biomass carbon; QBS-ar = soil biological quality index. The treatments include: NPK = nitrogen–phosphorous–potassium; HM = horse manure; C 50/50 = compost with 50% wood sawdust + 50% wet waste; C 10/90 = compost with 10% wood sawdust + 90% wet waste; VC 50/50 = vermicompost with 50% wood sawdust + 50% wet waste; VC 10/90 = vermicompost with 10% wood sawdust + 90% wet waste along with an unfertilized control CTR (control) = soil without fertilizers.

4. Discussion

4.1. Effects of Fertilizers on Soil Quality and QBS-ar Index

The results demonstrate that organic waste-derived amendments significantly improved soil quality across chemical, biochemical, and biological dimensions, in agreement with the soil quality framework proposed by Bünemann et al. [9], which emphasizes the integration of multiple indicators to assess soil functionality under different management practices. These findings are consistent with current soil health frameworks emphasizing the integration of multiple indicators to assess soil functionality under different management practices, as recently reported by Li et al. [32].

Organic amendments, particularly vermicompost 10/90, produced the strongest improvements in organic carbon, organic matter, cation exchange capacity (CEC), and C/N ratio, confirming their effectiveness in enhancing soil fertility in Mediterranean systems [8,33]. The increase in CEC reflects enhanced humification and the accumulation of negatively charged functional groups associated with organic inputs [34,35]. The concurrent increase in electrical conductivity (EC) likely reflects greater ion availability following organic matter mineralisation, indicating improved nutrient availability, although potential long-term salinity effects should be considered under repeated applications.

Biochemical and microbiological indicators revealed a strong stimulation of soil metabolic activity. Dehydrogenase activity (DHA), fluorescein diacetate hydrolase (FDA), and microbial biomass carbon (MBC) increased significantly under compost and especially vermicompost treatments, confirming that organic substrates promote microbial growth and enzymatic processes involved in nutrient cycling [7,11]. The strong correlations among OC, OM, DHA, and MBC highlight the central role of organic carbon in regulating microbial activity and soil functionality. PCA results further supported these findings, showing that organic treatments were associated with coordinated improvements in chemical and biological indicators, whereas NPK primarily stimulated microbial activity without enhancing soil organic matter or structural properties.

The QBS-ar index confirmed the positive effects of organic amendments on soil faunal communities. Higher QBS-ar values under compost and vermicompost treatments indicate improved habitat quality and increased microarthropod diversity, consistent with previous studies highlighting the sensitivity of soil fauna to organic matter inputs [36–38]. Enhanced microbial and enzymatic activity may also have contributed to the increased QBS-ar values by improving habitat quality and resource availability for soil microarthropods. In contrast, NPK resulted in lower QBS-ar values than the unfertilised control, suggesting potential negative effects of mineral fertilisation on soil biodiversity, likely due to reduced organic matter inputs and altered soil conditions [36].

4.2. Broccoli Quality

Broccoli quality was strongly influenced by fertilisation regime, reflecting the close relationship between soil conditions and crop nutritional composition. Organic amendments, particularly vermicompost 10/90, significantly enhanced both primary metabolites (proteins and carbohydrates) and secondary metabolites (phenolics, flavonoids, vitamins).

The increase in antioxidant compounds suggests that organic fertilisation promotes metabolic pathways involved in secondary metabolite synthesis, likely through improved nutrient balance and enhanced soil biological activity [39,40]. Comparable increases in antioxidant compounds and phenolic content following organic fertilisation have also been reported in vegetable crops grown under sustainable management systems [39,41]. In contrast, NPK fertilisation mainly supported primary metabolism, with limited effects on antioxidant compounds, indicating that mineral inputs alone may not stimulate secondary metabolism to the same extent [41].

PCA confirmed these patterns, showing clear associations between organic treatments and nutraceutical traits, whereas NPK was more closely related to primary metabolic parameters. These results indicate that improvements in soil biological and biochemical properties are directly linked to enhanced crop nutritional quality.

4.3. Multi-Indicator Assessment of Soil Quality

The multi-indicator approach provided a comprehensive assessment of soil functionality, highlighting the importance of integrating chemical, biological, and faunal indicators. This approach aligns with current soil quality assessment frameworks, which emphasize the need for multidimensional evaluation to capture ecosystem responses to management practices [9,32]. The spider plot clearly showed that compost and vermicompost treatments, particularly VC 10/90, promoted a coordinated improvement across chemical, biochemical, microbiological, and faunal soil quality indicators. In contrast, NPK and control treatments exhibited more limited and unbalanced responses, mainly associated with isolated increases in selected parameters rather than a comprehensive enhancement of soil functionality. These findings demonstrate that organic amendments improve not only individual soil properties, but also the functional integration among soil biological, biochemical, and chemical processes, thereby contributing to greater soil resilience and ecosystem stability [9,32].

The effectiveness of the multi-indicator framework was further supported by PCA and correlation analyses, which revealed strong positive relationships among organic carbon accumulation, microbial biomass, enzymatic activities, cation exchange capacity, and QBS-ar values. Similar integrated responses following organic amendment application have recently been reported by Li et al. [32], Huang et al. [35], and Gallese et al. [37], highlighting the importance of combining biological and biochemical indicators for a more realistic assessment of soil ecological functionality.

Overall, the multi-indicator framework proved effective in identifying treatment-specific responses and in capturing the synergistic effects of organic amendments on soil functionality. The combined evaluation of chemical, biochemical, microbiological, and faunal indicators provided a more comprehensive interpretation of soil ecosystem responses under different fertilisation regimes and further supports the use of integrated soil quality assessment approaches for sustainable soil management in Mediterranean agroecosystems [9,32,36–38].

5. Conclusions

This study demonstrates that compost and vermicompost derived from organic waste can significantly improve soil functionality and crop quality under Mediterranean agroecosystem conditions. Organic amendments enhanced soil chemical fertility, microbial biomass, enzymatic activity, and soil faunal diversity, confirming the importance of biologically active organic inputs for restoring degraded or low-organic-matter soils. Among the tested treatments, vermicompost 10/90 showed the most consistent and integrated positive effects across chemical, biochemical, microbiological, and faunal indicators, highlighting the importance of substrate composition in determining soil responses.

In contrast, mineral fertilisation (NPK) mainly stimulated selected biochemical processes without producing comparable improvements in soil organic matter accumulation, soil biodiversity, or overall ecological functionality. These findings suggest that mineral fertilisation alone may be insufficient to support long-term soil resilience and biological stability in Mediterranean environments.

The observed improvements in soil quality were closely associated with enhanced broccoli nutritional and nutraceutical traits, including antioxidant compounds, vitamins,

proteins, and carbohydrates. This confirms the strong relationship between soil biological functionality and crop nutritional quality and supports the potential role of waste-derived organic amendments in sustainable food production systems.

The multi-indicator approach adopted in this study proved effective for capturing the complexity of soil ecosystem responses and identifying coordinated interactions among chemical, biochemical, microbiological, and faunal processes. The integration of complementary indicators allowed a more comprehensive interpretation of treatment effects compared with the use of single soil parameters alone.

However, the present study was based on short-term observations under site-specific Mediterranean field conditions, and therefore the results should be interpreted within these experimental limitations. Although organic amendments significantly improved several indicators of soil quality, repeated long-term applications may alter soil C:N dynamics, nutrient availability, salinity, and organic matter turnover, potentially influencing long-term soil fertility and crop performance.

Future research should therefore focus on long-term field experiments aimed at evaluating the agronomic stability, environmental sustainability, and ecological impacts of repeated compost and vermicompost applications under different pedoclimatic conditions. Additional studies investigating the mechanistic relationships among organic matter composition, microbial processes, soil biodiversity, and crop metabolic responses would further improve the understanding of how waste-derived amendments regulate soil functionality and agricultural sustainability.

Author Contributions: Conceptualization, A.M. (Adele Muscolo), M.O. and L.S.; methodology, F.M.; A.M. (Angela Maffia); software, L.S.; validation, A.M. (Adele Muscolo), and C.M.; formal analysis, F.M. and A.M. (Angela Maffia); investigation, S.B. data curation, F.M. and A.M. (Angela Maffia); writing—original draft preparation, M.O. and L.S.; writing—review and editing, A.M. and E.A.; visualization, M.O., L.S.; project administration, A.M. (Adele Muscolo); funding acquisition, A.M. (Adele Muscolo). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Agritech National Research Center and received funding from the European Union Next-Generation EU (PIANO NAZIONALE DI RIPRESA E RESILI-ENZA (PNRR)—MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4—D.D. 1032 17 June 2022, CN00000022).

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Telo Da Gama, J. The Role of Soils in Sustainability, Climate Change, and Ecosystem Services: Challenges and Opportunities. *Ecologies* **2023**, *4*, 552–567. [CrossRef]
2. Nguyen, T.P.L.; Virdis, S.G.P. Climate Variability and Future Responses of Agricultural Systems in Mediterranean Region. *Farming Syst.* **2025**, *3*, 100153. [CrossRef]
3. Wu, H.; Cheng, X.; Zhou, Y.; Zhou, G.; Zhang, H.; Fang, X.; Shi, Y. Feedbacks between Vegetation and Soil in Global Forest Degradation: A Meta-Analysis. *Ecol. Indic.* **2026**, *185*, 114738. [CrossRef]
4. Elshaikh, A.; Mabrouki, J. Impact of Agricultural Soil Degradation on Water and Food Security. In *Artificial Intelligence Systems in Environmental Engineering*; CRC Press: Boca Raton, FL, USA, 2024; pp. 13–24.
5. Miransari, M. (Ed.) *Soil Nutrients; Environmental Health—Physical, Chemical and Biological Factors*; Nova Science Pub Inc.: New York, NY, USA, 2012.
6. Muscolo, A.; Marra, F.; Canino, F.; Maffia, A.; Mallamaci, C.; Russo, M. Growth, Nutritional Quality and Antioxidant Capacity of Lettuce Grown on Two Different Soils with Sulphur-Based Fertilizer, Organic and Chemical Fertilizers. *Sci. Hortic.* **2022**, *305*, 111421. [CrossRef]

7. Wang, F.; Zhang, Y.; Su, Y.; Wu, D.; Xie, B. Pollutant Control and Nutrient Recovery of Organic Solid Waste by Earthworms: Mechanism and Agricultural Benefits of Vermicomposting. *J. Environ. Chem. Eng.* **2024**, *12*, 112610. [CrossRef]
8. Gerke, J. The Central Role of Soil Organic Matter in Soil Fertility and Carbon Storage. *Soil Syst.* **2022**, *6*, 33. [CrossRef]
9. Bünemann, E.K.; Bongiorno, G.; Bai, Z.; Creamer, R.E.; De Deyn, G.; De Goede, R.; Fleskens, L.; Geissen, V.; Kuyper, T.W.; Mäder, P.; et al. Soil Quality—A Critical Review. *Soil Biol. Biochem.* **2018**, *120*, 105–125. [CrossRef]
10. Chaudhry, H.; Vasava, H.B.; Chen, S.; Saurette, D.; Beri, A.; Gillespie, A.; Biswas, A. Evaluating the Soil Quality Index Using Three Methods to Assess Soil Fertility. *Sensors* **2024**, *24*, 864. [CrossRef] [PubMed]
11. Semenov, M.V.; Zhelezova, A.D.; Ksenofontova, N.A.; Ivanova, E.A.; Nikitin, D.A.; Semenov, V.M. Microbiological Indicators for Assessing the Effects of Agricultural Practices on Soil Health: A Review. *Agronomy* **2025**, *15*, 335. [CrossRef]
12. FAO. *Agricultural Biodiversity: FAO Multifunctional Character of Agriculture and Land*; FAO: Rome, Italy, 1999.
13. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical: Rockville, MD, USA, 2005.
14. Bouyoucos, G.J. Hydrometer Method Improved for Making Particle Size Analyses of Soils1. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
15. Mehlich, A. Rapid Determination of Cation and Anion Exchange Properties and pHe of Soils. *J. Assoc. Off. Agric. Chem.* **1953**, *36*, 445–457. [CrossRef]
16. Box, J.D. Investigation of the Folin-Ciocalteau Phenol Reagent for the Determination of Polyphenolic Substances in Natural Waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
17. von Mersi, W.; Schinner, F. An Improved and Accurate Method for Determining the Dehydrogenase Activity of Soils with Iodonitrotetrazolium Chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
18. Beck, T. Die Messung Der Katalaseaktivität von Böden. *Z. Für Pflanzenernähr. Bodenkd.* **1971**, *130*, 68–81. [CrossRef]
19. Dick, R.P.; Breakwell, D.P.; Turco, R.F. Soil Enzyme Activities and Biodiversity Measurements as Integrative Microbiological Indicators. In *Methods for Assessing Soil Quality*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 1997; pp. 247–271.
20. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An Extraction Method for Measuring Soil Microbial Biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
21. Walkley, A.; Black, I. An Examination of the Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
22. Bano, R.; Roy, S. Extraction of Soil Microarthropods: A Low Cost Berlese- Tullgren Funnels Extractor. *Int. J. Fauna Biol. Stud.* **2016**, *2*, 14–17.
23. Angelini, P.; Fenoglio, S.; Isaia, M.; Jacomini, C.; Migliorini, M.; Morisi, A. Biomonitoraggio. In *Tecniche di Biomonitoraggio del Suolo*; ARPA Piemonte: Turin, Italy, 2002.
24. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. [CrossRef]
25. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant Activity of Some Algerian Medicinal Plants Extracts Containing Phenolic Compounds. *Food Chem.* **2006**, *97*, 654–660. [CrossRef]
26. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
27. Barreca, D.; Bellocco, E.; Caristi, C.; Leuzzi, U.; Gattuso, G. Flavonoid Composition and Antioxidant Activity of Juices from Chinotto (Citrus × Myrtifolia Raf.) Fruits at Different Ripening Stages. *J. Agric. Food Chem.* **2010**, *58*, 3031–3036. [CrossRef]
28. Prieto, P.; Pineda, M.; Aguilar, M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. *Anal. Biochem.* **1999**, *269*, 337–341. [CrossRef]
29. Davies, S.H.R.; Masten, S.J. Spectrophotometric Method for Ascorbic Acid Using Dichlorophenolindophenol: Elimination of the Interference Due to Iron. *Anal. Chim. Acta* **1991**, *248*, 225–227. [CrossRef]
30. Kruger, N.J. The Bradford Method For Protein Quantitation. In *The Protein Protocols Handbook*; Walker, J.M., Ed.; Humana Press: Totowa, NJ, USA, 2009; pp. 17–24.
31. Hedge, J.E.; Hofreiter, B.T.; Whistler, R.L. *Carbohydrate Chemistry*; Academic Press: New York, NY, USA, 1962; Volume 17, pp. 71–80.
32. Li, Y.; Zhao, H.; Liu, J.; Chaonan, C.; Yuxuan, G. A Framework for Selecting and Assessing Soil Quality Indicators for Sustainable Soil Management in Waste Dumps. *Sci. Rep.* **2024**, *14*, 8491. [CrossRef]
33. Sharma, S.; Lishika, B.; Shubham; Kaushal, S. Soil Quality Indicators: A Comprehensive Review. *Int. J. Plant Soil Sci.* **2023**, *35*, 315–325. [CrossRef]
34. Mahajan, N.C.; Mrunalini, K.; Prasad, K.S.K.; Naresh, R.K.; Sirisha, L. Soil Quality Indicators, Building Soil Organic Matter and Microbial Derived Inputs to Soil Organic Matter under Conservation Agriculture Ecosystem: A Review. *Int. J. Curr. Microbiol. Appl. Sci.* **2019**, *8*, 1859–1879. [CrossRef]
35. Huang, R.; Li, W.; Niu, M.; Hu, B. Organic Farming to Improve Soil Quality and the Functional Structure of Soil Microbial Communities. *Agriculture* **2025**, *15*, 1381. [CrossRef]

36. Menta, C.; Remelli, S. Soil Health and Arthropods: From Complex System to Worthwhile Investigation. *Insects* **2020**, *11*, 54. [CrossRef]
37. Gallese, F.; Gismero-Rodriguez, L.; Govednik, A.; Giagnoni, L.; Lumini, E.; Suhadolc, M.; Vaccari, F.P.; Maienza, A. Soil microarthropods as tools for monitoring soil quality: The QBS-AR Index in three European agroecosystems. *Agriculture* **2025**, *15*, 89. [CrossRef]
38. Remelli, S.; Menta, C. Soil biological quality across land-use gradients: An arthropod-based index from a comprehensive literature review. *Agric. Ecosyst. Environ.* **2026**, *404*, 110375. [CrossRef]
39. González-Coria, J.; Lozano-Castellón, J.; Jaime-Rodríguez, C.; Olmo-Cunillera, A.; Laveriano-Santos, E.P.; Pérez, M.; Lamuela-Raventós, R.M.; Puig, J.; Vallverdú-Queralt, A.; Romanyà, J. The Effects of Differentiated Organic Fertilization on Tomato Production and Phenolic Content in Traditional and High-Yielding Varieties. *Antioxidants* **2022**, *11*, 2127. [CrossRef]
40. Sindesi, O.; Mashece, W.; Silwana, S.; Sigidi, M. The Role of Nitrogen Fertilisation in Enhancing Growth, Yield, and Secondary Metabolite Production in Medicinal Plants: A Review. *DOAJ* **2025**, *134*, 183–199.
41. Antonious, G. The Impact of Organic, Inorganic Fertilizers, and Biochar on Phytochemicals Content of Three Brassicaceae Vegetables. *Appl. Sci.* **2023**, *13*, 8801. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Chapter 6 — General Discussion and Conclusions

6.1 Synthesis and Comparison of Results

The overall set of results presented in this thesis delineates a coherent framework in which soil quality emerges as the structural and functional dimension underpinning fertility, ecological resilience, and agricultural productivity in Mediterranean agroecosystems. This centrality arises not merely from nutrient accumulation but, more importantly, from the soil's capacity to sustain the biological and biochemical processes governing the cycling of matter and energy. Although Chapters 3, 4, and 5 detail the experimental evidence supporting this framework, Chapter 2 elucidates how humified organic matter and its associated bioactive compounds act as pivotal regulators of soil structure, microbial activity, and soil–plant interactions.

Chapter 2 describes humic substances as highly reactive components endowed with a dynamic, aggregated structure, and a marked capacity to interact with mineral surfaces and microbial communities. Their functions, including enzymatic modulation, enhanced nutrient availability, stimulation of root growth, and plant stress protection, form the theoretical basis for interpreting the results observed in the experimental chapters. Indeed, the organic amendments used in the experiments, enriched in humified fractions, produced simultaneous improvements in OC, OM, CEC, MBC, DHA, FDA, and edaphic biodiversity (QBS-ar), confirming the strong link between the nature of organic matter and overall soil functioning.

Chapter 3 shows how compost composition directly influences trajectories of edaphic regeneration. The compost, with a higher lignocellulosic component and a greater humification degree, enhanced stable organic matter fraction, cation exchange capacity, and enzymatic activities associated with long-term oxidative processes. The compost, rich in readily biodegradable material, triggered a more intense microbial response, with marked increases in MBC, EC, soil moisture, and actinomycete abundance. Although the operational dynamics of the two composts differ, one promoting organic matter stabilisation, the other promoting rapid mineralisation, both substantially improved soil

biological and functional quality, with positive repercussions on crops. Plants fertilized with compost exhibited higher photosynthetic efficiency, improved nutritional status, and superior biometric traits, demonstrating the direct link between soil quality and plant performance.

Chapter 4 further consolidated this framework by showing that organic-recycling-based fertilizers not only enhanced soil ecosystem functioning but also significantly improved the nutritional and nutraceutical quality of crops. The increases in soil organic carbon, microbial biomass, and key soil metabolic enzymes affected broccoli quality with a great increase in vitamin C, vitamin E, phenolics acids, flavonoids, carbohydrates, and proteins. The PCA of biochemical parameters confirmed that RecOrgFert PLUS fertilization drives plant secondary metabolism towards the synthesis of antioxidant compounds, underscoring the importance of soil processes in determining food quality. These findings suggest that biologically active and structurally stable soils constitute a prerequisite for producing foods with high nutraceutical value.

Chapter 5 integrated the results into a multi-indicator framework capable of capturing the intrinsically multidimensional nature of soil quality. The joint evaluation of chemical, functional, biochemical, microbiological, and faunal indicators revealed systemic patterns that would be difficult to detect using traditional single-parameter approaches. Compost and vermicompost treatments (particularly formulations with higher moist organic fractions such as VC 10/90) produced broad and balanced profiles in spider plots and formed distinct clusters in PCA analyses. By contrast, mineral NPK fertilization produced limited effects restricted to specific biochemical aspects, without improving soil organic structure, edaphic biodiversity, or microbial vitality. The control and the initial soil (To) remained representative of degraded Mediterranean soils, with uniformly low values across all indicators.

The integrated analysis of experimental evidence indicates that soil regeneration cannot be achieved through unidimensional inputs but requires the simultaneous activation of chemical, microbiological, enzymatic, and faunal processes. Compost, vermicompost, and innovative bio-based fertilizers were effective in re-establishing these functions, the soil's ecological integrity, defined by well-structured organic matter, an active microbial network, sustained biochemical functionality, and renewed soil biodiversity. Such

conditions are essential not only for maintaining agricultural productivity but also for enhancing the nutraceutical quality of crops and ensuring the long-term resilience of Mediterranean agroecosystems.

6.2 Scientific Contributions of the Thesis

6.2.1 Methodological Innovations

The integrated multi-scale approach presented in this thesis represents a significant methodological advancement in soil quality assessment. By combining chemical, biochemical, microbiological, and faunal indicators and integrating them through multivariate statistical tools (e.g., PCA) and synthetic graphical models (e.g., spider plots), this framework enables a systemic interpretation of soil ecosystem functioning. Unlike traditional analytical schemes that rely on single parameters, this approach provides a comprehensive understanding of the complex soil responses to organic fertilizers. Its innovative strength lies in the ability to link processes often treated as independent, such as mineralisation, microbial activity, carbon turnover, carbon sequestration, nutrient availability, and soil biodiversity, demonstrating that they operate as a coordinated network within a complex ecological system. The methodological framework developed here constitutes a transferable model applicable across diverse Mediterranean agricultural contexts, enhancing the diagnostic power of soil analyses and improving the interpretability of agroecological dynamics.

6.2.2 Multifactorial Indicators of Soil Quality

A particularly significant contribution of this thesis concerns the development of a truly multifactorial approach to soil quality, capable of integrating chemical, functional, biochemical, and microbiological dimensions with the faunal component, still too often marginalised in agronomic research. The combined use of indicators such as OC, OM, C/N ratio, CEC, soil moisture, EC, enzymatic activities (DHA, FDA), and microbial biomass (MBC) enabled an accurate delineation of soil response trajectories to organic fertilizers, highlighting the complex and integrated nature of edaphic processes.

The most innovative aspect, however, lies in the inclusion of the QBS-ar index, a fauna-based indicator grounded in the structure of soil microarthropod communities and offering a deeper ecological interpretation of the soil system. Unlike chemical or microbial indicators, QBS-ar reflects soil habitat quality, capturing variations in structure, porosity,

resource availability, and microenvironmental stability. Its sensitivity to management changes and its ability to integrate cumulative medium to long-term effects make it a particularly valuable tool for interpreting soil resilience.

In this study, QBS-ar proved to be a highly discriminating indicator: RecOrgFert PLUS, compost, and vermicompost produced marked increases in faunal quality, revealing more mature, stable, and functionally active soils. In contrast, mineral fertilizers yielded lower values, suggesting that while they support certain biochemical aspects, they do not recreate ecological conditions favourable to the development of soil fauna. Integrating QBS-ar within a multifactorial analytical framework therefore enhances the diagnostic capacity of soil assessment, allowing the detection not only of punctual improvements but also of the broader regenerative trajectory of the soil system. The full set of indicators adopted in this thesis emerges as an advanced tool for diagnosing soil quality and monitoring the long-term effects of agronomic practices, providing a robust foundation for the sustainable management of Mediterranean agroecosystems.

6.2.3 Impacts on Mediterranean Agroecology

This thesis also provides a significant contribution to Mediterranean agroecology by demonstrating that organic fertilizers and innovative products derived from agro-industrial residues can regenerate degraded soils while simultaneously improving the nutritional quality of crops. Compost, vermicompost, and sustainable fertilizers (such as RecOrgFert PLUS) proved capable of enhancing not only traditional edaphic parameters but also microbial vitality, enzymatic functionality, and soil biodiversity, critical components for rebuilding ecological resilience under adverse climatic conditions.

The impact of these fertilizers extends beyond agronomic performance: they promote carbon sequestration, improve soil structural stability, reduce dependency on chemical inputs, and transform organic waste into high-value agronomic resources. Collectively, these processes contribute to the development of genuinely circular, climate-smart agricultural systems capable of supporting food production with elevated nutraceutical value. In this way, the thesis provides robust scientific evidence to support agricultural policies and practices that integrate environmental sustainability, production efficiency, and the valorisation of residual biomass characteristic of Mediterranean regions.

6.3 Practical and Policy Implications for Organic and Circular Agriculture

The evidence produced in this thesis has direct relevance for agricultural management and for the development of policy strategies aimed at enhancing the sustainability of Mediterranean agroecosystems. The emergence of compost, vermicompost, and RecOrgFert PLUS, derived from agro-industrial residues, as tools capable of simultaneously regenerating the chemical, biological, enzymatic, and faunal components of soil demonstrates that fertility can no longer be conceptualised merely as nutrient availability but must instead be understood as an overarching ecological property. Systematic adoption of highly humified organic matrices therefore represents a concrete lever for improving soil ecological functioning and ensuring stable yields in an increasingly variable and stressful climatic context.

From a management perspective, the use of bio-based fertilizers enhances aggregate stability, improves cation exchange capacity, increases microbial biomass and soil biodiversity, and contributes to the development of production systems that are more resilient to the hydric and thermal stresses typical of Mediterranean environments. Integrating these tools into organic farming practices strengthens the potential to drastically reduce dependency on synthetic mineral inputs while simultaneously promoting food nutritional quality and the ecological sustainability of the agricultural system.

At policy level, these findings align closely with European strategies for ecological transition, particularly the EU Soil Strategy for 2030, the Green Deal, the Farm to Fork Strategy, and the Circular Economy Action Plan, all of which prioritise reductions in chemical inputs, biodiversity protection, and the valorisation of organic resources. The empirical demonstration of the effectiveness of biological fertilizers and organic-recycling products highlights the need for supportive policies promoting high-quality compost production, sustainable waste management, and certification systems based on integrated soil-quality indicators, including faunal parameters such as the QBS-ar index.

In this perspective, Mediterranean agriculture can serve as a privileged testing ground for genuinely circular models, in which waste is transformed into resources, fertility is built through biological processes, and soil quality becomes the cornerstone of future agronomic policies.

6.4 Future Research Perspectives

Although this thesis provides a significant advancement in understanding the processes that regulate soil quality and the effectiveness of bio-based sustainable fertilizers, several avenues for further investigation emerge from the findings. A first direction concerns the need to evaluate the long-term effects of bio-based fertilizers on microbial and faunal communities and detritus chain. Multi-year studies would help clarify the persistence of the observed benefits and identify potential ecological thresholds or saturation points.

Additional developments relate to the refinement of the multi-indicator framework, particularly through the integration of QBS-ar with other soil-fauna indicators and with metrics derived from automated analytical techniques (machine learning, computer vision, in situ sensors). A more comprehensive diagnostic system would enable more precise monitoring of soil ecological status and allow the early detection of signals of degradation or regeneration.

The growing availability of organic-recycling fertilizers underscores the need to develop dynamic agroecological simulation models capable of predicting the combined effects of these matrices on fertility, productivity, and carbon balance at farm scale. Such tools could support more informed agronomic decision-making and contribute to the design of agricultural systems able to withstand the impacts of climate change while maintaining high food quality and the ecological resilience of soils.

Appendix- Additional papers

Sustainable Soil Management: The Dynamic Impact of Combined Use of Crop Rotation and Fertilizers from Agri-Food and Sulfur Hydrocarbon Refining Processes Wastes

Angela Maffia[1], Federica Marra [1], Mariateresa Oliva[1], Santo Battaglia[1], Carmelo Mallamaci [1] and Adele Muscolo [1]*

[1]Agriculture Department, Mediterranea University, 89124 Reggio Calabria, Italy;

*Correspondence: amuscolo@unirc.it;

Land

https:// doi.org/10.3390/land14061171

Received: 28 April2025

Revised: 22 May2025

Accepted: 27 May2025

Published: 29 May2025

land

MDPI

Article

Sustainable Soil Management: The Dynamic Impact of Combined Use of Crop Rotation and Fertilizers from Agri-Food and Sulfur Hydrocarbon Refining Processes Wastes

Angela Maffia, Federica Marra, Mariateresa Oliva, Santo Battaglia, Carmelo Mallamaci and Adele Muscolo *

Agriculture Department, Mediterranea University, 89124 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); federica.marra@unirc.it (F.M.); mariateresa.oliva@unirc.it (M.O.); santo.battaglia@unirc.it (S.B.); carmelo.mallamaci@unirc.it (C.M.)

* Correspondence: amuscolo@unirc.it; Tel.: +39-0965-169-4364

Abstract: Sustainable agriculture increasingly relies on strategies that improve soil fertility while reducing the environmental footprint of chemical inputs. The primary objective of this research was to disentangle the individual and combined effects of crop rotation and fertilization on soil quality. This study aimed to determine whether the effectiveness of fertilization was modified by rotational practices—exploring whether these interactions were additive, antagonistic, or synergistic. This study assessed the impact of two-year open-field crop rotations—broccoli–tomato and broccoli–pepper—combined with organic and mineral fertilization on soil chemical and biological properties. Treatments included sulfur bentonite enriched with orange waste (SBO), horse manure (HM), mineral fertilizer (NPK), and an unfertilized control (CTR). Soil samples were collected after each crop cycle and analyzed for enzymatic activities (fluorescein diacetate hydrolase, dehydrogenase, catalase), microbial biomass carbon (MBC), organic matter, total nitrogen, and macro- and micronutrient content. The results showed that organic amendments, particularly SBO and HM, significantly increased microbial activity, MBC, and nutrient availability compared to NPK and CTR. Organic treatments also led to a reduction in soil pH (−12%) and a more balanced ionic profile, enhancing soil biological fertility across both rotations. By contrast, the NPK treatments favored higher nitrate and chloride concentrations (3.5 and 4.6 mg * g^{-1} dw, respectively) but did not improve biological indicators. Improvements were more pronounced in the second crop cycle, suggesting the cumulative benefits of organic amendments over time. These findings highlight the potential of combining organic fertilization with crop rotation to enhance soil health and support long-term sustainability in horticultural systems.

Keywords: soil fertility; organic amendments; crop rotation; microbial biomass; enzyme activity; sustainable agriculture

check for updates

Academic Editor: Lúcia Barão

Received: 28 April 2025
Revised: 22 May 2025
Accepted: 27 May 2025
Published: 29 May 2025

Citation: Maffia, A.; Marra, F.; Oliva, M.; Battaglia, S.; Mallamaci, C.; Muscolo, A. Sustainable Soil Management: The Dynamic Impact of Combined Use of Crop Rotation and Fertilizers from Agri-Food and Sulfur Hydrocarbon Refining Processes Wastes. *Land* **2025**, *14*, 1171. https://doi.org/10.3390/land14061171

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Global food production must increase by 70% to meet the demands of a growing population, projected to reach 9.7 billion by 2050 [1]. This has led to the intensification of agriculture to boost productivity, driven by the widespread use of mineral fertilizers and plant protection products. However, this intensification has also contributed to a rise in soil degradation, threatening soil health. Agriculture plays a crucial role in natural resource management, but its expansion and intensification can negatively impact environmental

protection and accelerate the degradation of agricultural lands [2,3]. In response, an ongoing debate has emerged regarding the need for improved fertilizer management and more sustainable soil nutrient use. This highlights the importance of integrating methods and practices that ensure adequate plant nutrition while maintaining long-term soil productivity. Sustainable soil management remains a fundamental challenge in modern agriculture, particularly in the face of growing environmental concerns and the need for efficient resource utilization. To maintain or to improve the fertility and productivity of the agricultural soil, many types of waste can be used, such as organic municipal waste, sewage sludge, waste from agricultural crops, manure from animals, and some types of industrial wastes, as a source of organic matter [4]. In previous works Muscolo et al. [5], Maffia et al. [6], and Panuccio et al. [7] used fertilizers produced from different agro-industrial wastes to improve crop yield and quality, and soil quality. The results evidenced at different extents a positive impact of all the fertilizers produced from wastes on the soil and crops. The integration of agro-industrial waste-derived fertilizers with crop rotation strategies has recently emerged as a promising approach to enhance soil fertility while reducing reliance on synthetic inputs. However, the dynamic interactions between soil amendments and cropping systems require thorough investigation to verify if their combination can be more suitable and productive than their single use, to optimize agronomic practices and ensure long-term sustainability. Crop rotation is a -time-honored agricultural practice known to improve soil health, manage pests and diseases, and enhance crop yields by diversifying the crops grown in succession on the same land [8]. By alternating crops with different nutrient requirements and rooting patterns, crop rotation helps to maintain soil nutrient balance and structure, thereby reducing the need for chemical fertilizers [9]. The utilization of agro-industrial wastes as fertilizers presents an eco-friendly alternative to conventional fertilization methods. Composting and vermicomposting agricultural and industrial wastes can improve both aboveground and belowground ecosystem services, contributing to sustainable agriculture [10]. Sulfur is a macronutrient essential for optimal plant growth and for enhancing resistance to soil-borne fungal and bacterial diseases [11]. In recent years, sulfur has gained renewed importance as a plant nutrient due to its declining availability in the soil. This reduction is attributed to the following: (1) the increased use of mono-ammonium and di-ammonium phosphate fertilizers, which contain less sulfur compared to traditional superphosphate; (2) the decreased atmospheric deposition of sulfur as a result of reduced industrial emissions; and (3) the adoption of higher-yielding crop varieties that extract greater amounts of sulfur from the soil during harvest [12].

Furthermore, sulfur is permitted in organic agriculture worldwide, making the recovery of sulfur from agricultural and industrial wastes an agronomically beneficial and environmentally sustainable strategy [13]. In the Calabria region of Southern Italy, research has shown that the application of sulfur bentonite combined with orange processing wastes—rich in valuable biomolecules such as nutrients and phenolic compounds—can improve soil fertility and promote eco-friendly agricultural practices [14–17]. Based on previous experiments, this study investigated the combined effects of crop rotation and the innovative fertilizer produced from agro-industrial wastes (sulfur bentonite plus orange waste) on soil quality and fertility. Conducted over two consecutive years, the research employed a rotational system alternating a winter crop (broccoli) with two different summer crops (tomato and pepper). Specifically, Calabrian broccoli, Big Rio tomato, and Topepo pepper were selected, as these are crops cultivated in Calabria with significant economic impact. The experiment was carried out in two distinct plots (1/2 hectare each) within the same area, each plot was further divided into multiple subplots to ensure statistical robustness. One plot featured a broccoli–pepper rotation, while the other employed a broccoli–tomato rotation. The primary objective of this research was to disentangle the

individual and combined effects of crop rotation and fertilization on soil quality, with particular attention to the influence of different summer crops grown in rotation with broccoli. The study aimed to determine whether the effectiveness of fertilization was modified by rotational practices—exploring whether these interactions were additive, antagonistic, or synergistic—and whether different rotational crops exerted distinct impacts on soil fertility parameters. Additionally, the research sought to evaluate how the integration of agro-industrial waste-derived fertilizers with diversified cropping systems could contribute to the long-term improvement of soil health and function. By analyzing a range of soil physical and chemical properties under varied crop and fertilization combinations, this study offers critical insights into soil–plant dynamics, informing carbon farming strategies and supporting the transition toward sustainable, resilient, and productive agricultural systems.

2. Materials and Methods

2.1. Fertilizer Production

Sulfur bentonite plus orange waste (called pastazzo) have been produced by SBS Steel Belt Systems srl in tablets of 3/4 mm, as described by Muscolo et al. [18,19]. Sulfur (S) was mixed with bentonite (B) and orange residues (O) coming from the food industry. Elemental S was the principal component of the fertilizer [20]. The fertilizer was tested for pathogens (*total coliforms*, *faecal coliforms*, *salmonella* spp., and *Escherichia coli*) and heavy metals to avoid a pollutant impact on the soil [21]. The results evidenced an absence of pathogens and heavy metals [20]. The composition of the sulfur bentonite plus orange waste and horse manure fertilizers is reported below, in Table 1.

Table 1. Chemical characteristic of sulfur bentonite plus orange waste and horse manure fertilizers. Data are the mean of three replications ± standard deviation. Different letters in the same row indicate, significant differences among the treatments (* Tukey's test, $p \leq 0.05$).

Chemical Properties	HM	SBO
pH	7.0 ^{a*} ± 0.1	6.8 ^{a} ± 0.2
EC (mS/cm)	12 ^{a} ± 1.1	1.3 ^{b} ± 0.9
Moisture (%)	86.7 ^{a} ± 3.2	nd ± 2.9
OC (%)	22.6 ^{a} ± 1.9	6.3 ^{b} ± 2.5
TN (%)	3.0 ^{a} ± 0.6	0.9 ^{b} ± 0.3
C/N	7.3 ^{a} ± 1.9	9.3 ^{a} ± 1.7
Na^+ (mg g^{-1} dw)	1.8 ^{a} ± 0.5	0.12 ^{b} ± 0.2
NH_4^+ (mg g^{-1} dw)	0.24 ^{a} ± 0.03	0.09 ^{b} ± 0.04
K^+ (mg g^{-1} dw)	3.2 ^{a} ± 2.3	1.32 ^{b} ± 2.6
Mg^{2+} (mg g^{-1} dw)	1.9 ^{a} ± 0.4	1.41 ^{b} ± 0.7
Ca^{2+} (mg g^{-1} dw)	2.3 ^{a} ± 0.7	1.1 ^{b} ± 1.0
Cl^- (mg g^{-1} dw)	3.8 ^{a} ± 0.5	0.11 ^{b} ± 0.6
PO_4^{3-} (g g^{-1} dw)	2.8 ^{a} ± 0.4	0.3 ^{b} ± 0.3

2.2. Open Field Experiment

Extensive open-field tests were conducted over a total area of 1 hectare to evaluate the effects of fertilizer application and seasonal vegetable crop rotation on soil properties. The experiment took place at Falcone Farm in San Lorenzo (Reggio Calabria). The soil is classified as Sandy–Clay–Loam, with an alkaline pH and a low organic matter content ranging from 0.7% to 1.6%. Before the fertilizer application, the soil in the subplots underwent comprehensive analysis, with the results reported in the Tables as T0. The 1-hectare field was divided into two plots of 0.5 hectares each, which were further subdivided into subplots. Four fertilizer treatments were applied on a nitrogenous base:

- SBO (476 kg S ha^{-1})—Sulfur-based amendment

- CTR (Control)—Unfertilized soil
- NPK (20/10/10) (476 kg S ha^{-1})—Chemical fertilizer
- HM (430 kg ha^{-1})—Horse manure as an organic amendment

The experiment was arranged in a randomized complete block design and three replicates of each treatment were conducted across three subplots, with six soil samples collected per subplot for each treatment. The experiment was conducted over two consecutive years, and the results represent the average of three independent trials. Throughout the study, irrigation was maintained at 70% of field capacity to support soil vitality. Soil water content was monitored using a direct-read soil pH/moisture meter (R181). The SBO application rate was selected based on the literature data, which suggests pure sulfur application rates ranging from 2200 kg S ha^{-1} to 3300 kg S ha^{-1}, depending on the soil texture [18,22]. Broccoli was placed in the soil in October and collected in February. After broccoli harvesting, one plot rotated with the Topepo red pepper, and the other one with the tomato Big Rio F1. The tomato and pepper were harvested in July. Soil was collected after the crop harvesting, dried and sieved at 2 mm except for microbial biomass C, and analyzed for their chemical and biological properties, as reported in the soil chemical analysis paragraph.

2.3. Soil Chemical Analysis

The following soil properties were detected. Soil texture with the hydrometer method [23]; electric conductibility (EC) in 1:5 soil/water suspension, after stirring at 15 rpm for 1 h was measured with a Hanna instrument conductivity meter; the pH was determined in soil/solution ratio 1:2.5 with a glass electrode. Organic carbon was detected using the Walkley–Black method [24]. Total nitrogen (TN) was assessed using the Kjeldahl method [25]. C/N was quantified as a carbon/nitrogen ratio. Water soluble phenols were extracted and analyzed as described by Kaminsky and Müller [26], and monomeric and polyphenols were determined by the Box method [27], using tannic acid as the standard. The concentration of water-soluble phenolic compounds was expressed as tannic acid equivalents (µg TAE g^{-1}D.W.). The cation exchange capacity was analyzed using the barium chloride method [28]. Cations and anions were detected using ion chromatography (DIONEX ICS-1100) [29].

2.4. Soil Biological Analysis

The microbial biomass carbon (MBC) was assessed using the chloroform fumigation–extraction procedure [30] on fresh soil. Fumigated and unfumigated soil sample extracts were used to detect the soluble organic C [24]. Fluorescein diacetate hydrolase (FDA) activity was determined according to the method of Adam and Duncan [31]. Dehydrogenase (DHA) activity was assessed using the von Mersi and Schinner method [32]. Catalase activity (CAT) was detected by assessing the absorbance during the transformation of H_2O_2 to oxygen and water [18]. The decrease in the absorbance was measured at 240 nm, using the extinction coefficient of 39.4 M^{-1} cm^{-1}. Protease activity was detected as reported in Muscolo et al. [18]. Urease activity was determined as described in Sidari et al. [33]. Ammonium concentrations were determined at 690 nm by using a calibration curve. The results are reported as µg N-NH_4 g^{-1} d^{-1} 3 h^{-1}.

2.5. Statistical Analysis

An analysis of variance was used for all the data sets. One-way ANOVA with Tukey's honestly significant difference tests for analyzing the effects of fertilizer and crop rotation on each of the parameters measured were used. The ANOVA and *t*-test were performed using SPSS software, version 29.0. The effects were significant at $p \leq 0.01$. To analyze the

relationships between the fertilizer, crop rotation, and soil parameters in the two different plots, a principal component analysis (PCA) was used.

3. Results

After the first cycle of the broccoli–tomato crop rotation, notable changes were observed in the soil chemical properties. The water content increased across all treatments compared to both the baseline control (initial soil conditions) and the unfertilized soil (Table 2). The soil pH decreased significantly only in the presence of SBO. Among the treatments, the SBO uniquely increased the concentration of water-soluble phenols (WSP), whereas the NPK was the only treatment that led to a significant reduction in WSP. Organic carbon (OC), total nitrogen (TN), cation exchange capacity (CEC), and microbial biomass carbon (MBC) increased across all treatments, including the unfertilized control (Table 2). Enzymatic activities, including fluorescein diacetate hydrolysis (FDA) and dehydrogenase (DHA), increased in the HM and SBO treatments relative to both controls. However, catalase (CAT) activity decreased with the SBO application (Figure 1). No significant differences were found in the lithium content. By contrast, potassium and magnesium levels rose compared to the control, while the calcium content declined in the soil treated with HM and SBO. Nitrate concentrations peaked with the NPK treatment, whereas sulfate concentrations were highest in the soil treated with SBO (Figure 2).

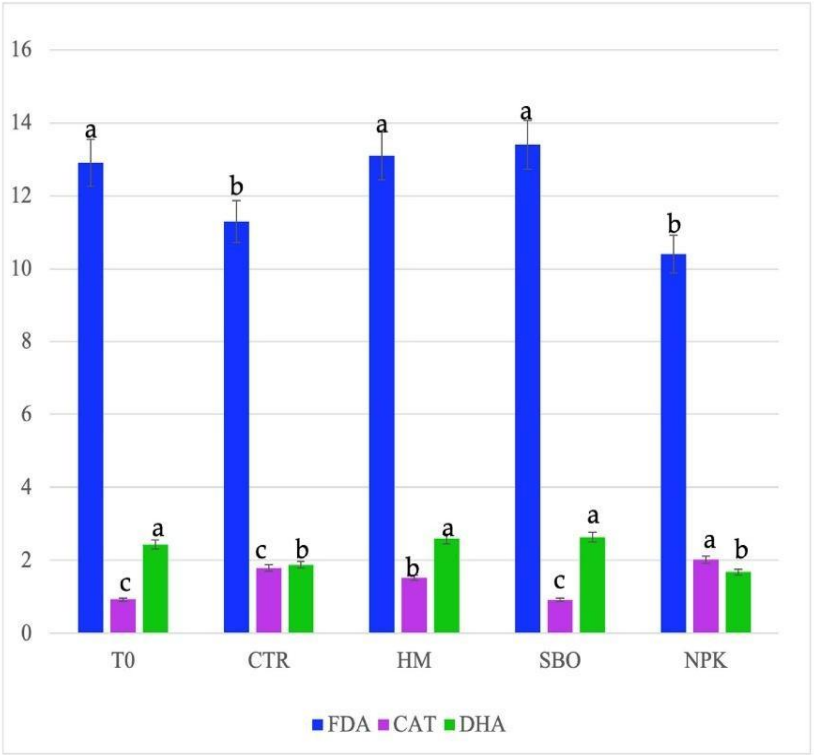


Figure 1. Fluorescein diacetate hydrolase (FDA, µg FDA/g d.s.), dehydrogenase (DHA, µg TTF/h/g d.s.), and catalase, (CAT, O_2/3 min/g d.s.) detected in soil after the second cycle of broccoli–tomato crop rotation. The data refers to the open field experiments carried on from October 2022 to July 2023. Soil samples were collected after the tomato harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

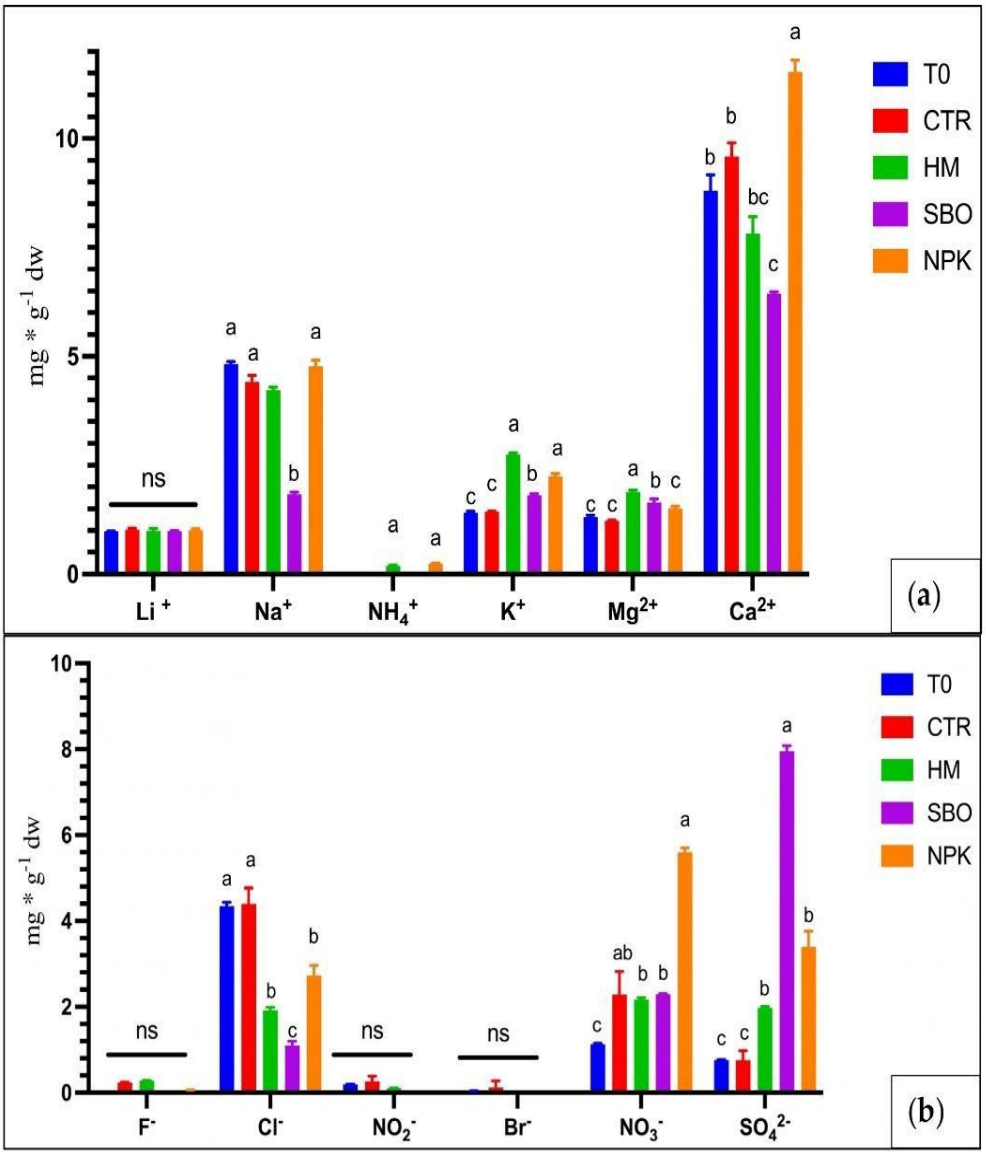


Figure 2. Cations (**a**) and anions (**b**) (mg * g^{-1} dw) in soil under the broccoli–tomato crop rotation. The first crop rotation cycle, from October 2022 to July 2023, with soil samples collected after the tomato harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$). n.s. = not significant ($p > 0.05$).

In the second cycle of the broccoli–tomato rotation, electrical conductivity (EC) increased compared to the initial conditions (T0), especially in the soil treated with SBO and NPK. The pH again decreased with the SBO application (Table 3), while WSP increased in all treatments, most markedly in the unfertilized soil. FDA and DHA activities, along with OC, TN, and MBC, increased in the HM and SBO treatments relative to controls. Conversely, the CAT activity declined (Figure 3).

Table 2. Chemical and biological properties of soil after the first crop cycle of the broccoli–tomato rotation (October 2022–July 2023), with samples collected after tomato harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). The measured variables include water content (WC, %), pH, electrical conductivity (EC, μS cm^{-1}), water-soluble phenols (WSP, μg TAE g^{-1} dry soil), organic carbon (OC, %), organic matter (OM, %), total nitrogen (TN, %), carbon-to-nitrogen ratio (C/N), cation exchange capacity (CEC, $cmol^{+}$ kg^{-1}), and microbial biomass carbon (MBC, μg C g^{-1}). Values are expressed as a mean ± standard deviation. Different letters within the same column indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

	WC (%)	pH	EC	WSP	OC	OM	TN	C/N	CEC	MBC
T0	7.8 ± 0.2^{b}	8.16 ± 0.1^{a}	100 ± 6^{e}	23.3 ± 3^{b}	0.73 ± 0.03^{d}	1.25 ± 0.1^{d}	0.09 ± 0.01^{b}	8.1 ± 0.2^{b}	13.4 ± 0.1^{b}	433 ± 8^{e}
CTR	8 ± 0.2^{b}	8.24 ± 0.2^{a}	171 ± 9^{d}	23.2 ± 2^{b}	1.40 ± 0.02^{c}	2.40 ± 0.3^{c}	0.10 ± 0.02^{b}	14.0 ± 0.2^{a}	15.2 ± 0.1^{b}	476 ± 10^{d}
HM	13 ± 0.2^{a}	8.19 ± 0.2^{a}	232 ± 10^{a}	22.4 ± 3^{b}	1.70 ± 0.04^{b}	2.92 ± 0.1^{b}	0.15 ± 0.01^{a}	11.3 ± 0.1^{a}	17.1 ± 0.2^{a}	752 ± 12^{b}
SBO	16 ± 0.2^{a}	7.99 ± 0.1^{a}	207 ± 8^{b}	28.8 ± 2^{a}	1.98 ± 0.1^{a}	3.40 ± 0.2^{a}	0.14 ± 0.02^{a}	14.1 ± 0.2^{a}	17.0 ± 0.2^{a}	788 ± 11^{a}
NPK	16 ± 0.2^{a}	8.38 ± 0.2^{a}	195 ± 9^{b}	19.5 ± 3^{b}	1.67 ± 0.07^{b}	2.87 ± 0.2^{b}	0.18 ± 0.02^{a}	9.2 ± 0.01^{b}	16.5 ± 0.02^{a}	611 ± 13^{c}

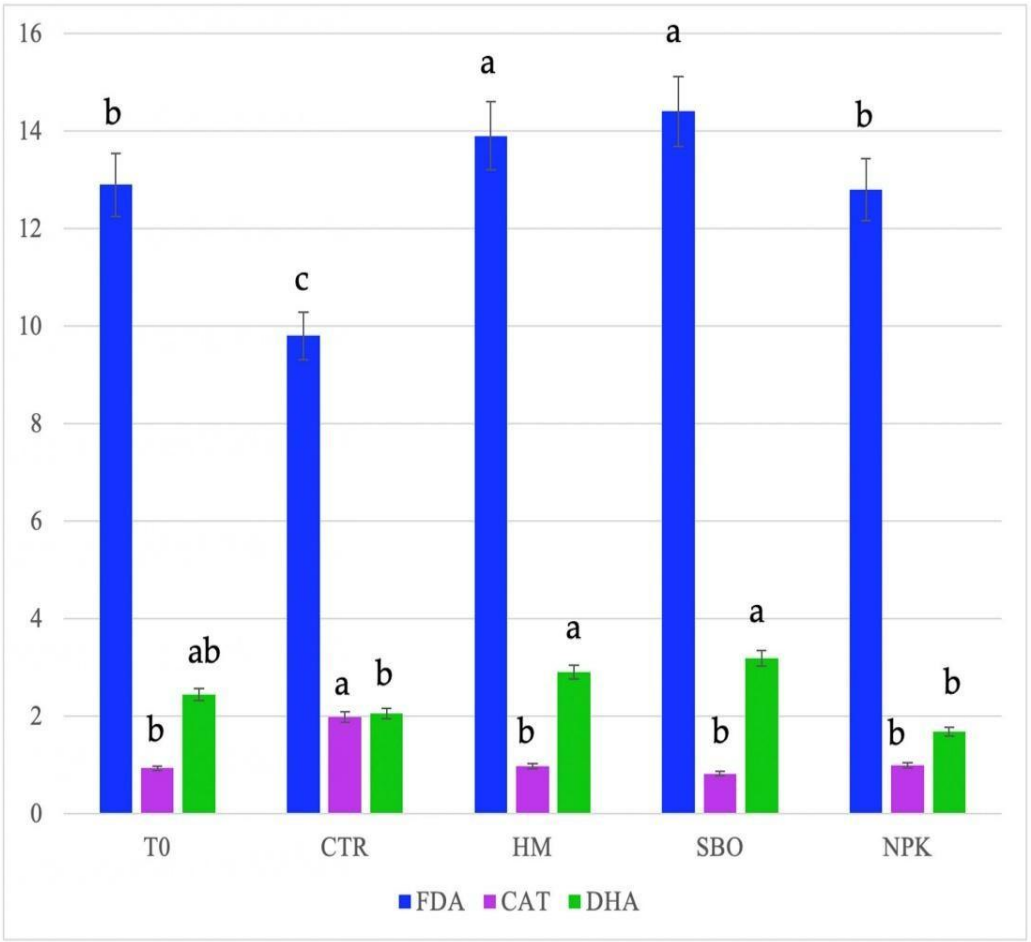


Figure 3. Fluorescein diacetate hydrolase (FDA, μg FDA/g d.s.), dehydrogenase (DHA, μg TTF/h/g d.s.), and catalase, (CAT, O_2/3 min/g d.s.) detected in soil after the second cycle of the broccoli–tomato crop rotation. The data refers to the open field experiments carried on from October 2022 to July 2023. Soil samples were collected after the tomato harvest. Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

Table 3. Chemical and biological properties of soil after the second crop cycle of the broccoli–tomato rotation (October 2022–July 2023), with samples collected after tomato harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). The measured variables include water content (WC, %), pH, electrical conductivity (EC, µS cm^{-1}), water-soluble phenols (WSP, µg TAE g^{-1} dry soil), organic carbon (OC, %), organic matter (OM, %), total nitrogen (TN, %), carbon-to-nitrogen ratio (C/N), cation exchange capacity (CEC, $cmol^{+}$ kg^{-1}), and microbial biomass carbon (MBC, µg C g^{-1}). Values are expressed as a mean ± standard deviation. Different letters within the same column indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

	WC (%)	pH	EC	WSP	OC	TN	C/N	OM	CEC	MBC
T0	7.85 ± 0.2^{c}	8.16 ± 0.2^{a}	100 ± 7^{d}	23.3 ± 2^{c}	0.73 ± 0.05^{d}	0.09 ± 0.001^{b}	8.1 ± 1^{d}	1.25 ± 0.05^{d}	13.4 ± 0.5^{c}	433 ± 15^{d}
CTR	11.0 ± 0.1^{b}	8.21 ± 0.1^{a}	217 ± 9^{c}	45.1 ± 6^{a}	1.62 ± 0.1^{c}	0.08 ± 0.002^{b}	20.2 ± 3^{a}	2.40 ± 0.1^{c}	15.7 ± 0.7^{a}	498 ± 14^{d}
HM	14.0 ± 0.7^{a}	7.89 ± 0.2^{ab}	220 ± 8^{c}	30.2 ± 4^{b}	2.14 ± 0.2^{ab}	0.19 ± 0.001^{a}	11.2 ± 1^{c}	3.68 ± 0.2^{a}	16.3 ± 0.5^{a}	952 ± 15^{b}
SBO	14.0 ± 0.6^{a}	7.50 ± 0.2^{b}	310 ± 11^{b}	27.9 ± 3^{b}	2.30 ± 0.2^{a}	0.17 ± 0.001^{a}	13.5 ± 2^{b}	3.95 ± 0.3^{a}	15.0 ± 1^{a}	988 ± 10^{a}
NPK	11.0 ± 0.8^{b}	8.29 ± 0.3^{a}	370 ± 9^{a}	25.0 ± 2^{c}	1.95 ± 0.1^{b}	0.17 ± 0.002^{a}	11.4 ± 1^{c}	3.35 ± 0.2^{b}	14.4 ± 0.4^{b}	0761 ± 11^{c}

When comparing the first and second crop cycles, soil properties, anions, and cations changed within each treatment. To integrate these multidimensional changes, a principal component analysis (PCA) was conducted using all chemical, biochemical, and ionic parameters measured across both crop cycles under the broccoli–tomato rotation. The biplot (Figure 4) shows the spatial distribution of treatments and variables along the first two principal components, which together explained 59.03% of the total variance (F1: 39.49%; F2: 19.54%). Samples from the second cycle (T02, CTR2, SBO2, HM2, NPK2) generally shifted toward the positive side of F1, closely associated with improved biological and fertility indicators, including MBC, DHA, FDA, EC, TN, and CEC. By contrast, untreated or initial-cycle samples, like T02 and CTR1, were positioned on the negative side of F1, in proximity to variables such as Cl^{-}, Br^{-}, $NO_2{}^{-}$, and Na^{+}, suggesting lower microbial activity and potential ionic accumulation. This multivariate visualization supports the observed trend of progressive soil improvement under organic treatments across successive crop cycles, distinguishing them from the mineral or unfertilized plots both chemically and biologically.

In the first broccoli–pepper crop rotation cycle, the SBO treatment led to a decrease in the soil pH and an increase in EC, WSP, FDA, OC, OM, and MBC, especially when compared to the NPK treatment and the two controls. The water content increased across all treatments compared to T0 and the control (Table 4).

Table 4. Chemical analysis of soil under the broccoli–pepper crop rotation. The first cycle refers to the first crop rotation cycle, from October 2022 to July 2023, with soil samples collected after the pepper harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). The measured variables include water content (WC, %), pH, electrical conductivity (EC, µS cm^{-1}), water-soluble phenols (WSP, µg TAE g^{-1} dry soil), organic carbon (OC, %), organic matter (OM, %), total nitrogen (TN, %), carbon-to-nitrogen ratio (C/N), cation exchange capacity (CEC, $cmol^{+}$ kg^{-1}), and microbial biomass carbon (MBC, µg C g^{-1}). Values are expressed as a mean ± standard deviation. Different letters within the same column indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

	WC (%)	pH	EC	WSP	OC	TN	C/N	OM	CEC	MBC
T0	7.85 ± 0.2^{c}	8.16 ± 0.3^{a}	100 ± 2.1^{c}	23.34 ± 0.6^{b}	0.73 ± 0.5^{c}	0.09 ± 0.5^{b}	8.1 ± 0.6^{c}	1.25 ± 0.1^{c}	13.4 ± 0.4^{b}	433 ± 4.6^{c}
CTR	9.3 ± 2.1^{b}	8.16 ± 0.5^{a}	188 ± 4.1^{b}	30.96 ± 0.7^{a}	1.48 ± 0.4^{b}	0.08 ± 0.1^{b}	18.5 ± 0.3^{a}	2.54 ± 0.7^{b}	14.1 ± 0.5^{b}	499 ± 3.4^{c}
HM	16.5 ± 1.2^{a}	8.14 ± 0.7^{a}	233 ± 2.3^{ba}	26.85 ± 0.5^{b}	1.93 ± 0.1^{b}	0.18 ± 0.1^{a}	10.7 ± 0.5^{b}	3.31 ± 0.2^{a}	18.2 ± 0.5^{a}	852 ± 4.4^{b}
SBO	16.5 ± 3.1^{a}	7.94 ± 0.9^{a}	275 ± 2.1^{a}	24.34 ± 2.1^{b}	2.16 ± 0.1^{a}	0.17 ± 0.2^{a}	12.7 ± 0.4^{ab}	3.71 ± 0.1^{a}	16.9 ± 0.7^{a}	988 ± 5.6^{a}
NPK	16.00 ± 3.4^{a}	8.38 ± 0.1^{a}	295 ± 3.4^{a}	19.55 ± 1.4^{c}	1.67 ± 0.3^{b}	0.18 ± 0.1^{a}	9.2 ± 0.6^{c}	2.87 ± 0.2^{b}	15.6 ± 0.8^{b}	735 ± 5.5^{b}

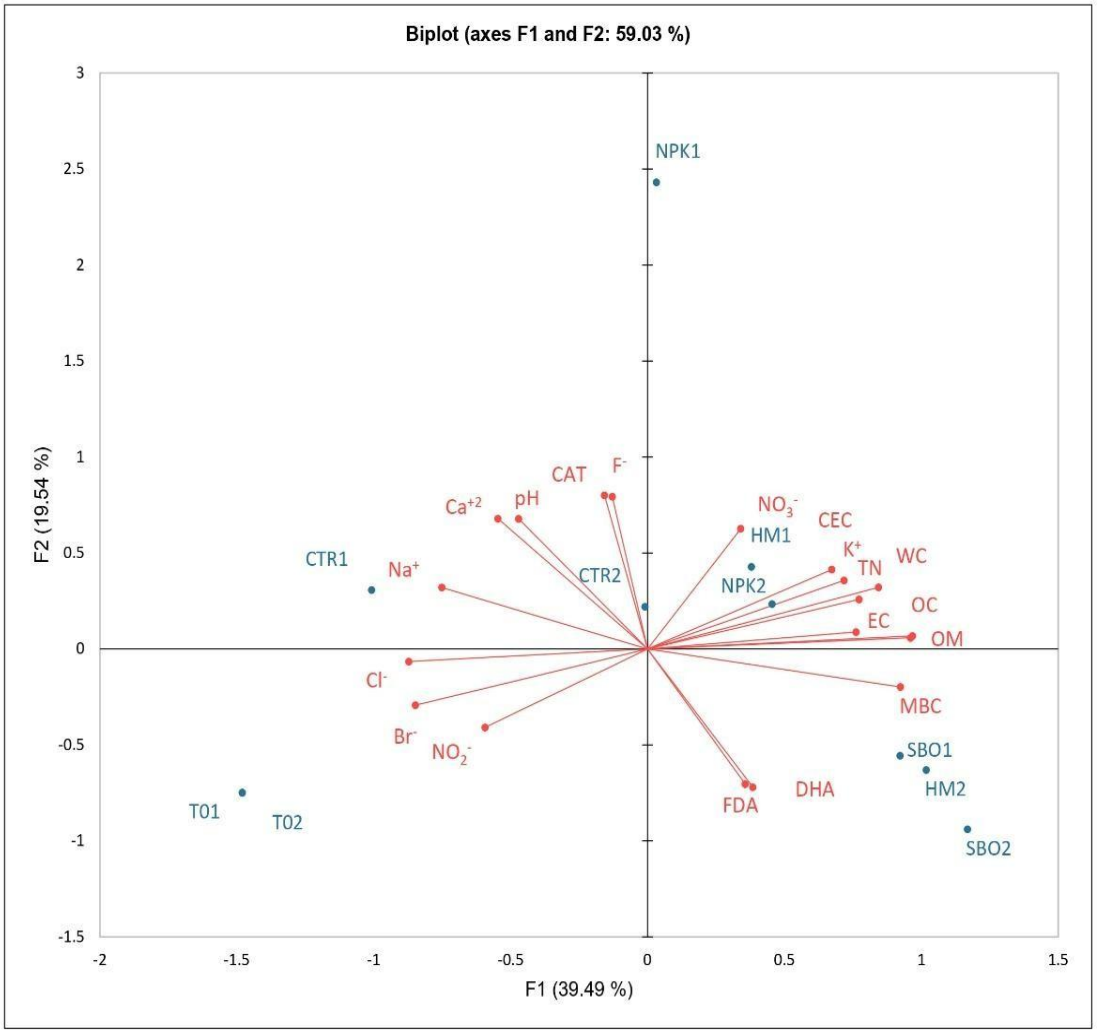


Figure 4. Principal component analysis (PCA) of chemical, biochemical, and ionic soil parameters under the broccoli–tomato crop rotation. The biplot represents soil samples collected after the first cycle (T01, CTR1, NPK1, HM1, SBO1) and the second cycle (T02, CTR2, NPK2, HM2, SBO2).

FDA activity, representing the overall microbial hydrolytic potential, was highest in the SBO-treated soil, followed closely by HM, while the lowest values were observed in the CTR and NPK treatments. DHA activity followed a similar pattern, peaking under the SBO treatment, indicative of enhanced microbial respiration and metabolic activity. By contrast, CAT activity, which reflects oxidative stress regulation, remained relatively low across all treatments, with only slight variations among them. The T0 and NPK treatments showed slightly higher CAT values compared to the organic amendments (Figure 5). The cation analysis revealed higher concentrations of K^+ and Mg^{2+} in the HM and SBO treatments, indicating improved nutrient availability from organic sources. Ca^{2+} was most abundant in the NPK and CTR treatments, while Na^+ levels were elevated in the T0 and mineral-fertilized soil (Figure 6a). Regarding anions, NO_3^- and Cl^- peaked under NPK, confirming the influence of synthetic fertilization. SO_4^{2-} and PO_4^{3-} were notably higher in the SBO-treated plots, reflecting sulfur and phosphorus contributions from the amendment (Figure 6b). Overall, organic treatments enriched the soil with key

macroelements while minimizing salt accumulation. This trend continued in the second cycle: The pH decreased in the HM and SBO treatments, while EC, OC, OM, TN, CEC, and MBC increased compared to both T0 and control (Table 5).

Table 5. Chemical analysis of soil under the broccoli–pepper crop rotation. The second cycle refers to the second crop rotation cycle, from October 2023 to July 2024, with soil samples collected after the pepper harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). The measured variables include water content (WC, %), pH, electrical conductivity (EC, µS cm^{-1}), water-soluble phenols (WSP, µg TAE g^{-1} dry soil), organic carbon (OC, %), organic matter (OM, %), total nitrogen (TN, %), carbon-to-nitrogen ratio (C/N), cation exchange capacity (CEC, $cmol^{+}$ kg^{-1}), and microbial biomass carbon (MBC, µg C g^{-1}). Values are expressed as a mean ± standard deviation. Different letters within the same column indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

	WC (%)	pH	EC	WSP	OC	TN	C/N	OM	CEC	MBC
T0	7.85 ± 0.1^{c}	8.16 ± 0.1^{a}	100 ± 1.5^{b}	23.34 ± 1.5^{b}	0.73 ± 0.1^{c}	0.09 ± 0.2^{a}	8.1 ± 0.7^{c}	1.25 ± 0.1^{c}	13.4 ± 1.2^{b}	433 ± 2.5^{c}
CTR	$10\,0 \pm 0.2^{b}$	8.25 ± 0.2^{a}	192 ± 2.5^{ab}	37.5 ± 0.5^{a}	1.54 ± 0.1^{b}	0.10 ± 0.3^{a}	15.4 ± 0.8^{a}	2.65 ± 0.3^{b}	13.9 ± 1.3^{b}	501 ± 2.7^{c}
HM	13.5 ± 0.2^{b}	7.97 ± 0.4^{a}	223 ± 2.7^{a}	25.50 ± 1.7^{b}	1.93 ± 0.2^{b}	0.16 ± 0.3^{a}	12.1 ± 1.1^{b}	3.31 ± 0.3^{a}	15.2 ± 1.6^{a}	879 ± 3.5^{a}
SBO	13.0 ± 0.3^{b}	7.81 ± 0.4^{a}	249 ± 3.5^{a}	32.50 ± 1.2^{a}	2.10 ± 0.2^{a}	0.13 ± 0.3^{a}	16.2 ± 1.2^{a}	3.61 ± 0.4^{a}	15.81 ± 1.2^{a}	955 ± 4.7^{a}
NPK	16.00 ± 0.3^{a}	8.38 ± 0.7^{a}	264 ± 2.3^{a}	19.55 ± 1.4^{c}	1.67 ± 0.3^{b}	0.18 ± 0.2^{a}	9.2 ± 1.2^{bc}	2.87 ± 0.1^{b}	15.6 ± 1.2^{a}	755 ± 5.6^{b}

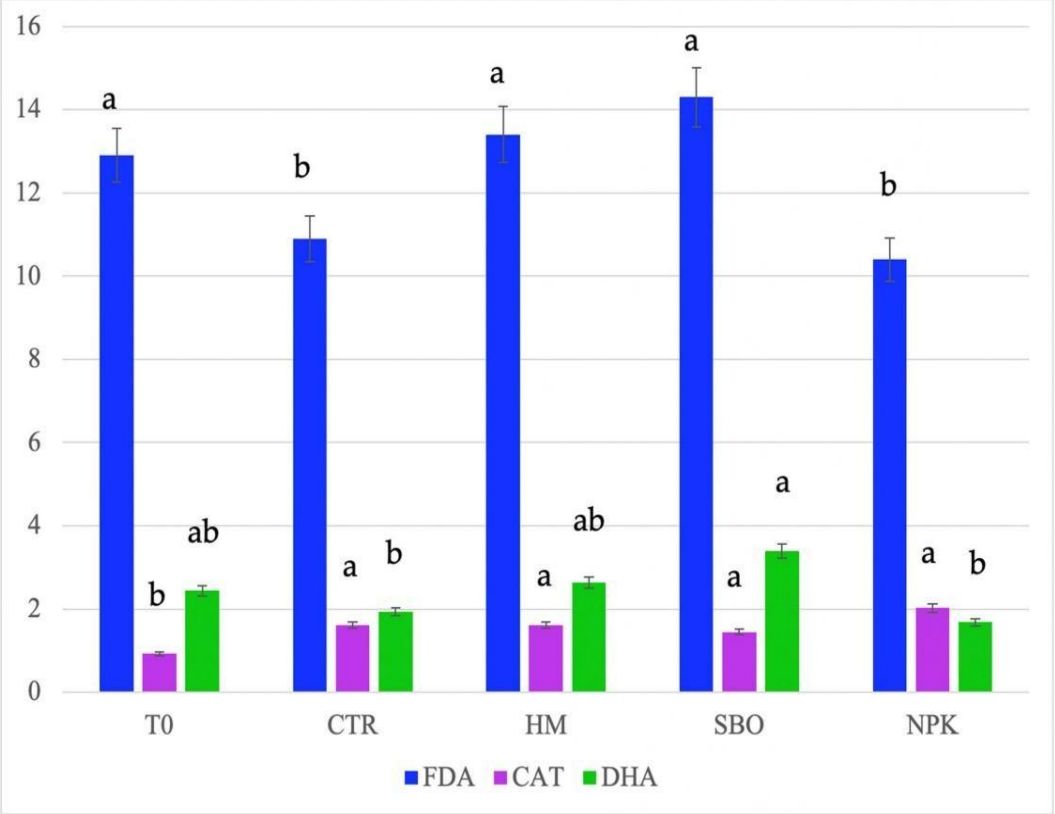


Figure 5. Fluorescein diacetate hydrolase (FDA, µg FDA/g d.s.), dehydrogenase (DHA, µg TTF/h/g d.s.), and catalase, (CAT, O_2/3 min/g d.s.) detected in soil after the first cycle of the broccoli–pepper crop rotation. The data refers to the open field experiments carried on from October 2022 to July 2023. Soil samples were collected after the tomato harvest. Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

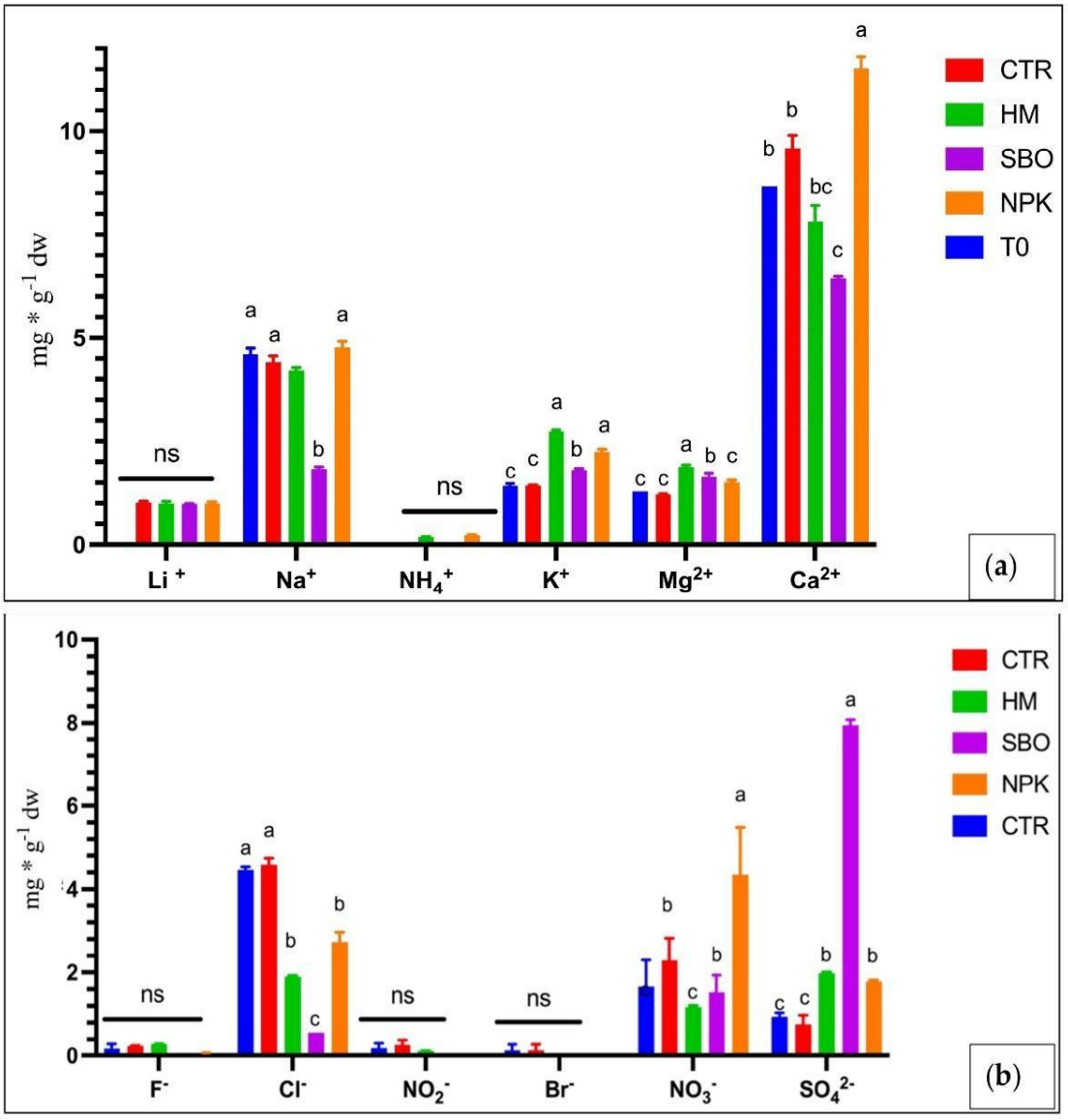


Figure 6. Cations (**a**) and anions (**b**) (mg * g^{-1} dw) in soil under the broccoli–pepper crop rotation. The first crop rotation cycle, from October 2022 to July 2023, with soil samples collected after the pepper harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$). n.s. = not significant ($p > 0.05$).

Enzymatic activities measured after the second cycle of the broccoli–pepper rotation (Figure 7) showed an overall increase compared to the first cycle. FDA activity remained highest in the HM and SBO treatments, indicating sustained microbial hydrolytic capacity, followed by the T0 and NPK treatments. DHA levels were also elevated under the SBO and HM treatments, confirming enhanced microbial respiration. CAT activity remained consistently low across all treatments, with minor differences; slightly higher values were recorded in the T0 and CTR treatments suggesting limited oxidative stress variation among

the treatments. Overall, the enzymatic profiles in the second cycle mirrored those of the first, with a general trend of increased microbial functional activity in organically amended soil. In the second cycle of the broccoli–pepper rotation, Ca^{2+} remained highest in the NPK-treated soil, while K^{+} and Mg^{2+} increased under the HM and SBO treatments, indicating enhanced nutrient input from organic amendments, while Na^{+} persisted at higher levels in T0, and NH_4^{+} stayed low across all treatments. Among anions, Cl^{-} and NO_3^{-} peaked in the NPK treatment, reflecting continued mineral fertilization. SO_4^{2-} and PO_4^{3-} were elevated in the SBO and HM treatments, supporting the influence of sulfur- and phosphorus-rich amendments. Minor ions, such as NO_2^{-} and Br^{-}, remained low, with slight increases in the mineral treatments. Overall, organic inputs improved nutrient availability, while the NPK treatment favored ion accumulation (Figure 8).

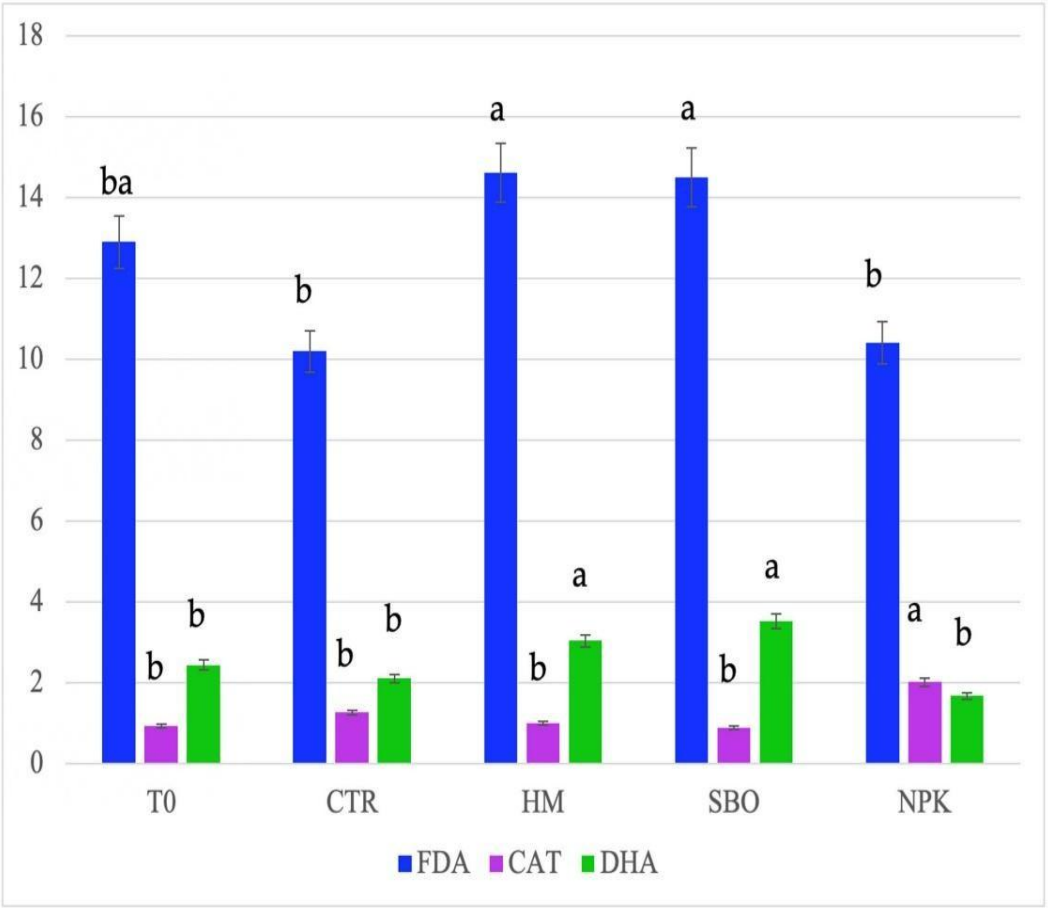


Figure 7. Fluorescein diacetate hydrolase (FDA. µg FDA/g d.s.), dehydrogenase (DHA. µg TTF/h/g d.s.), and catalase. (CAT. O_2/3 min/g d.s.) detected in soil after the second cycle of the broccoli–pepper crop rotation. The data refers to the open field experiments carried on from October 2022 to July 2023. Soil samples were collected after the tomato harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$).

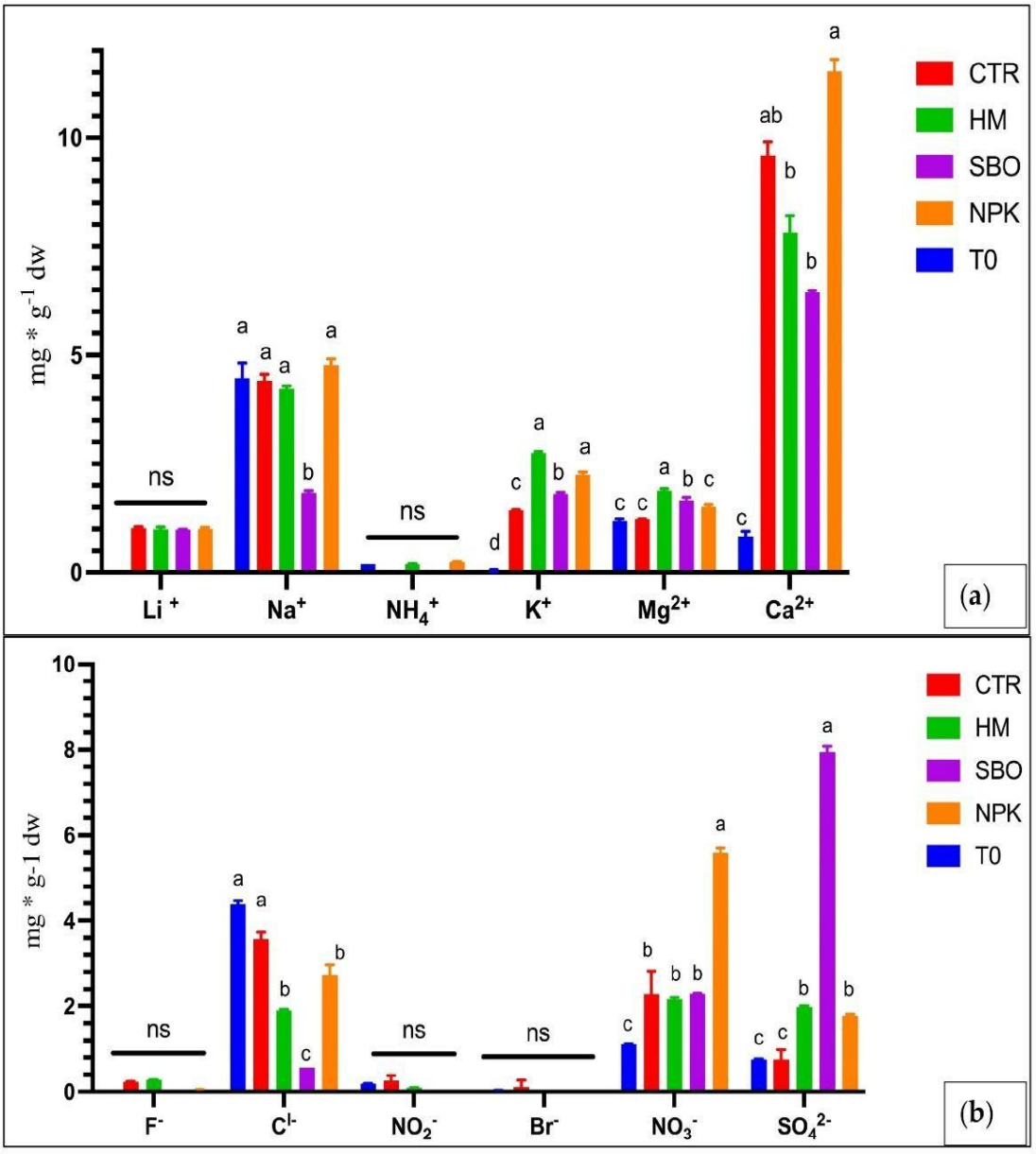


Figure 8. Cations (**a**) and anions (**b**) (mg * g^{-1} dw) in soil under the broccoli–pepper crop rotation. The second crop rotation cycle, from October 2023 to July 2024, with soil samples collected after the pepper harvest. The treatments include T0 (initial soil), CTR (unfertilized control), HM (horse manure), SBO (sulfur bentonite plus orange waste), and NPK (mineral fertilizer). Different letters indicate statistically significant differences among the treatments according to Tukey's test ($p < 0.05$). n.s. = not significant ($p > 0.05$).

To integrate the multivariate effects of treatments over time, a principal component analysis (PCA) was conducted using chemical, biochemical, and ionic soil parameters measured across both crop cycles under the broccoli–pepper rotation. The biplot (Figure 9) illustrates the distribution of treatments and active variables along the first two principal components, which together explained 62.80% of the total variance (F1: 41.38%; F2: 21.43%).

Samples from the second cycle (T02, CTR2, SBO2, HM2, NPK2) displayed a clear separation from first-cycle samples, with SBO2 and HM2 positioned on the far right of F1, closely associated with MBC, OM, DHA, SO_4^{2-}, and CEC, indicating enhanced microbial and nutrient status under organic management. First-cycle treatments, such as SBO1 and HM1, were also positively associated with fertility-related variables like EC, TN, and WC. Conversely, T02 and CTR1/CTR2 were located on the negative side of F1, clustering with Cl^-, NO_2^-, Na^+, and Mg^{2+}, suggesting lower biological activity and possible ionic accumulation. NPK1 and NPK2 were grouped along the upper right quadrant, aligning with NO_3^-, NH_4^+, and CAT, reflecting a mineral nitrogen-driven soil profile. This PCA confirms the distinct and progressive enrichment of soil biological and chemical properties under organic amendments, while distinguishing the mineral- and non-treated plots along contrasting multivariate trajectories.

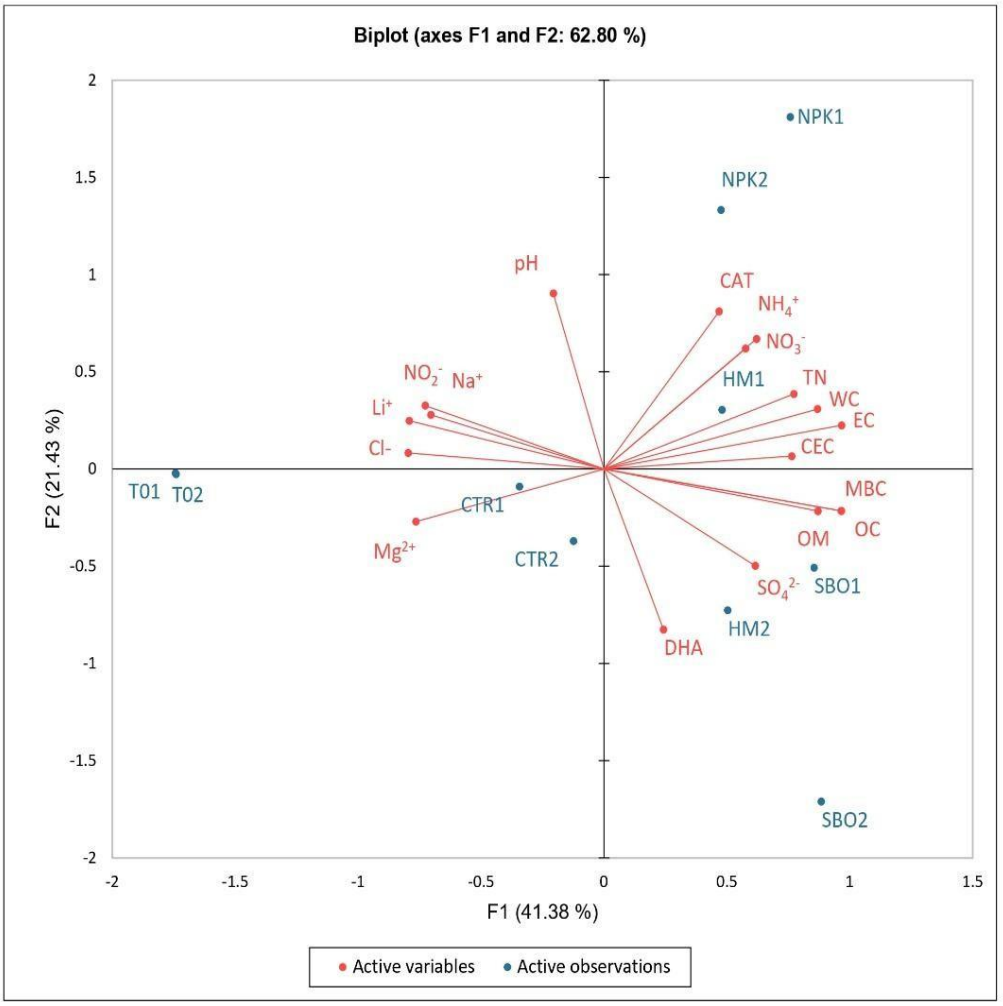


Figure 9. Principal component analysis (PCA) of chemical, biochemical, and ionic soil parameters under the broccoli–pepper crop rotation. The biplot represents soil samples collected after the first cycle (T01, CTR1, NPK1, HM1, SBO1) and the second cycle (T02, CTR2, NPK2, HM2, SBO2).

To assess whether the combined effects of fertilization and crop rotation influenced soil functioning, a correlation matrix was computed using all chemical and biochemical

variables measured across both crop cycles (Table 6). This analysis revealed several significant relationships that clarify how different management strategies shape soil fertility dynamics. The correlation matrix highlights strong positive relationships among organic matter (OM), organic carbon (OC), total nitrogen (TN), cation exchange capacity (CEC), and microbial biomass carbon (MBC), indicating enhanced fertility with organic inputs. Enzymatic activities (FDA, DHA) correlate positively with MBC and negatively with pH and catalase (CAT), suggesting increased microbial activity under acidified conditions. Electrical conductivity (EC) and water content (WC) also show positive associations with key fertility indicators.

Table 6. Correlation matrix (Pearson (n − 1) among soil chemical, biochemical, and ionic variables measured across both crop rotation cycles and all fertilization treatments. Values in bold are different from 0 with a significance level, alpha = 0.005.

Variables	WC	pH	EC	WSP	FDA	CAT	DHA	OC	TN	C/N	OM	CEC	MBC
WC	**1**	−0.313	0.446	0.042	0.144	0.049	0.242	0.773	0.760	0.141	0.779	0.800	0.718
pH	−0.313	**1**	−0.242	−0.199	−0.730	0.615	**−0.901**	−0.506	−0.326	−0.132	−0.522	−0.064	−0.715
EC	0.446	−0.242	**1**	0.225	0.209	−0.106	−0.020	**0.820**	0.638	0.334	**0.810**	0.269	0.704
WSP	0.042	−0.199	0.225	**1**	−0.274	0.146	0.080	0.275	−0.265	**0.842**	0.159	0.116	0.062
FDA	0.144	−0.730	0.209	−0.274	**1**	**−0.901**	0.781	0.266	0.399	−0.384	0.345	−0.099	0.642
CAT	0.049	0.615	−0.106	0.146	**−0.901**	**1**	−0.632	−0.056	−0.180	0.394	−0.120	0.394	−0.416
DHA	0.242	**−0.901**	−0.020	0.080	0.781	−0.632	**1**	0.267	0.183	−0.035	0.291	0.097	0.573
OC	0.773	−0.506	**0.820**	0.275	0.266	−0.056	0.267	**1**	0.767	0.426	**0.992**	0.640	**0.892**
TN	0.760	−0.326	0.638	−0.265	0.399	−0.180	0.183	0.767	**1**	−0.225	**0.821**	0.490	**0.839**
C/N	0.141	−0.132	0.334	**0.842**	−0.384	0.394	−0.035	0.426	−0.225	**1**	0.320	0.361	0.089
OM	0.779	−0.522	**0.810**	0.159	0.345	−0.120	0.291	**0.992**	**0.821**	0.320	**1**	0.625	**0.922**
CEC	0.800	−0.064	0.269	0.116	−0.099	0.394	0.097	0.640	0.490	0.361	0.625	**1**	0.481
MBC	0.718	−0.715	0.704	0.062	0.642	−0.416	0.573	**0.892**	**0.839**	0.089	**0.922**	0.481	**1**

Three-way ANOVA (Table 7) revealed that fertilization had a significant effect on all soil chemical and biological variables ($p < 0.001$). Several parameters, including MBC, CAT, DHA, FDA, and OM, also exhibited significant two- and three-way interactions.

Table 7. The *p*-values from three-way ANOVA testing the effects of fertilization, crop rotation, and year on soil chemical and biological variables. Significance levels: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s. = not significant ($p > 0.05$).

Variable	Fertilizer	Fertilizer × Rotation	Fertilizer × Year	Fertilizer × Year × Rotation	Rotation	Year	Year × Rotation
C/N	***	***	***	***	n.s	***	***
CAT	***	***	***	***	***	***	n.s
CEC	***	***	***	***	*	***	n.s
DHA	***	***	***	**	***	***	*
EC	***	n.s	***	***	n.s	***	***
FDA	***	***	***	***	n.s	***	n.s
MBC	***	**	**	***	***	***	***
OC	***	*	*	**	n.s	***	***
OM	***	***	**	***	n.s	***	***
TN	***	n.s	***	***	n.s	n.s	***
WC	***	***	***	***	***	***	***
WSP	***	***	***	***	***	***	***
pH	***	n.s	*	n.s	n.s	***	n.s

Main effects of the year were significant for most variables, especially for microbial and enzymatic indicators such as FDA and WC. By contrast, the rotation alone had limited effects, but its interaction with other factors (notably Year × Rotation) was significant for selected variables including OC, TN, and WC.

4. Discussion

Scientific studies have investigated the individual and combined effects of crop rotation and organic amendments on soil health and crop productivity illuminating that both practices offer significant benefits, with their effectiveness varying in respect to specific agricultural contexts and objectives. Crop rotation has been shown to enhance soil microbial biomass and diversity. A global synthesis revealed that crop rotation increased soil microbial biomass carbon (MBC) by 13.43% and microbial biomass nitrogen (MBN)

by 15.84%, compared to continuous monoculture systems [34]. Additionally, Shannon's diversity index, a measure of microbial diversity, was significantly elevated by 7.68% under crop rotation practices [35]. These improvements were attributed to the varied root exudates and organic matter inputs from different crops, which create a more hospitable environment for diverse microbial communities [36]. These findings can also explain the increase in organic matter in the plots amended with the inorganic fertilizer NPK. The application of organic amendments instead directly added organic matter to the soil, thereby enhancing the soil organic carbon (SOC) stocks. Research carried on in UK arable systems, comparing diversified crop rotations and organic amendments have confirmed our results, evidencing significant SOC accumulation in the upper layer of the soil (0–30 cm) amended with organic fertilizers [37]. The increase in soil organic carbon (SOC) has been consistently associated with enhanced soil physical and chemical properties, including improved aggregate stability, increased water-holding capacity, and greater nutrient retention and availability. Furthermore, the application of organic amendments significantly stimulated soil biological activity, as evidenced by elevated enzymatic activities, such as dehydrogenase and FDA. These enzymatic processes are key indicators of microbial functioning and nutrient turnover, thereby playing a pivotal role in sustaining nutrient cycling and promoting long-term soil health and fertility [38]. Our results demonstrated that fertilization exerts a more dominant influence on soil fertility and ecosystem functioning than crop rotation alone. Specifically, the control plots subjected to crop rotation without fertilization exhibited significantly lower levels of soil organic matter, microbial biomass carbon (MBC), and enzymatic activities, indicating that the absence of nutrient input limits biological and biochemical soil functions. This trend was further supported by observations in the plots treated with mineral fertilizers (NPK), which also showed reduced concentrations of organic matter, MBC, and enzymatic activity compared to the organically amended plots. These findings highlighted the limited capacity of crop rotation by itself to sustain soil biological quality in the absence of external nutrient inputs. A principal component analysis (PCA) further reinforced this conclusion by revealing a strong positive correlation between dehydrogenase activity, MBC, and organic matter with the HM and SBO treatments, underscoring the central role of fertilization in enhancing microbial-driven soil processes. While both crop rotation and organic fertilization independently contributed to the improved soil health, they contribute to varying extents. Organic fertilization, particularly with biologically enriched amendments, tends to have a more immediate and pronounced effect on microbial activity, soil organic carbon (SOC) accumulation, and nutrient availability. By contrast, crop rotation—especially when incorporating diverse species or extended ley periods—contributes more gradually by enhancing root diversity, soil structure, and long-term nutrient cycling. For example, integrating multi-year grass–clover leys within crop rotations, combined with compost fertilization, has been shown to significantly increase SOC levels compared to either practice alone [39]. This combination leverages both the continuous input of organic residue and the structural and functional benefits of diverse cropping, ultimately leading to improved soil fertility, biological activity, and agroecosystem resilience. The changes observed in the soil properties across two crop rotation cycles (broccoli–tomato and broccoli–pepper) highlighted the interactive effects of both crop identity and fertilization strategies. While the crop species (tomato vs. pepper) significantly influenced microbial and nutrient dynamics, the type of fertilizer applied played a central role in modulating soil health indicators, such as pH, enzymatic activity, organic matter, and nutrient availability [40]. These findings reinforce the complexity of plant–soil–microbe–fertilizer interactions in agroecosystems, and their importance in shaping sustainable soil management practices [41]. The fertilizer type had a marked influence on the direction and magnitude of soil property changes. Notably, SBO and HM were

more effective than NPK or the unfertilized control in enhancing microbial activity, organic carbon levels, and nutrient cycling. Organic fertilizers, such as SBO and HM, significantly reduced the soil pH, particularly with SBO, and with the effect being more pronounced in the broccoli–pepper system in the second cycle of both rotations. This acidification is likely due to the sulfur added with SBO that is well known to decrease the pH. Souri and Sayadi [42] demonstrated the efficiency of sulfur bentonite granules in decreasing the soil pH and improving the uptake of nutrient elements by the crop cultivated in calcareous soil. Additionally, the decrease in the soil pH in the presence of HM and mostly of SBO can be ascribed also to the oxidation of organic compounds, nitrification of ammonium, and excretion of organic acids by roots due to an increase in microorganisms enhanced by the high carbon input from organic amendments and by sulfur, as already demonstrated by other authors [43]. The mild acidification of the NPK fertilization is consistent with its targeted nutrient supply and minimal organic content. The increase in EC, most prominent under NPK, followed by SBO, reflected the accumulation of soluble ions released during the mineralization of organic matter (in SBO) or supplied directly via inorganic salts (in NPK). There is a difference in the soil electric conductivity also between tomato and pepper in rotation with broccoli, which may be due to the different absorption capacity and amount of soil ions between the two varieties. Although higher EC can indicate improved nutrient availability, it may also signal potential risks of salinity build up by fertilizer application [44].

All fertilization treatments, including the unfertilized control, even if at a different extent, led to an increase in the soil organic carbon (OC) and total nitrogen (TN), suggesting a cumulative contribution from plant residues and microbial biomass. However, the greatest increases were recorded under SBO and HM, due to their direct organic inputs and their role in stimulating microbial carbon use efficiency and residue incorporation [45]. By contrast, NPK showed a limited capacity to build the SOC, confirming the well-established notion that inorganic fertilization alone lacks the capacity to sustain long-term improvements in soil organic matter [46]. Interestingly, while SBO initially increased TN, a decline was observed in the second cycle of broccoli–pepper, likely due to the combination of rapid nitrogen mineralization and high uptake by pepper plants. This points to a potential mismatch between nitrogen release and crop demand, emphasizing the need for synchronized fertilization strategies. CEC increased under all treatments except for NPK, with the most significant enhancement seen in the SBO- and HM-treated soil. This is attributable to the high humified material supported by high value of C/N which contribute to greater ion retention and buffering capacity [44]. The decline in exchangeable calcium, sodium, and magnesium, particularly under organic treatments, may result from enhanced leaching or competitive uptake by plants and microorganisms. Organic amendments significantly promoted enzymatic activities (FDA and DHA), confirming their role in stimulating microbial metabolism through increased substrate availability [47]. The application of SBO, in particular, elevated water-soluble phenols (WSP), which serve as carbon-rich energy sources for microbes and can enhance the degradation of complex organic matter [48].

However, catalase (CAT) activity declined under SBO, suggesting a possible oxidative stress response or a shift in microbial community composition, possibly away from catalase-producing taxa [47]. While a decrease in catalase activity is often associated with oxidative stress and microbial imbalance, in certain contexts it may also reflect a stabilization or maturation of the soil microbial community. For example, in soil where organic matter inputs are reduced and overall microbial respiration rates decline, the generation of reactive oxygen species (ROS), like hydrogen peroxide, may also decrease. As a result, the microbial demand for antioxidant enzymes, such as catalase, diminishes, leading to lower catalase activity. In this case, the decline could indicate a lower oxidative burden in the soil

environment, potentially associated with more stable redox conditions or a shift toward microbial communities that maintain equilibrium with reduced enzymatic antioxidant requirements. The contrasting effects of SBO—increasing overall microbial activity while reducing CAT—highlight the complex, compound-specific impacts of organic fertilizers on microbial ecology. The NPK application led to increased nitrate and ammonium concentrations, indicating high immediate nutrient availability. However, its effect on microbial activity, organic carbon, and CEC was limited, aligning with the findings that inorganic fertilization often supports short-term productivity but fails to improve the long-term soil health [49]. The reduction in WSP under the NPK treatment further underscores its limited support for microbial substrate pools. While the type of fertilizer shaped the baseline soil changes, the subsequent crop species modulated the degree and direction of those changes through rhizosphere-driven mechanisms. For instance, pepper, particularly under SBO, intensified the decline in TN and pH, and amplified enzymatic activities, compared to tomato. This suggests that pepper's rhizosphere environment is more dynamic, with higher exudate-driven microbial turnover, possibly due to greater dependence on AMF symbioses and root-induced nutrient solubilization [50]. The synergistic effect of organic amendments with pepper roots likely accelerated nutrient cycling processes, explaining the elevated nitrate and sulfate concentrations. Conversely, tomato's effects appeared more moderate and buffered, promoting a more stable microbial environment, with less dramatic shifts in nitrogen and pH.

The strong and consistent effect of fertilization across all variables supports the central role of nutrient input in determining soil fertility status. However, the significant interaction effects—especially those involving year and crop rotation—highlight that fertilization outcomes are not static, but depend on the temporal and biological context.

Microbial and enzymatic indicators, such as MBC, CAT, DHA, and FDA, were especially sensitive to interactions, suggesting that microbial dynamics are modulated by both management practices and the crops involved. The effect of rotation was more evident in the interaction terms than as a main factor, which suggests that crop sequence influences soil processes indirectly, by modifying the plant–microbe interactions over time.

These results emphasize the need to consider fertilization strategies in combination with crop rotation and seasonal dynamics to optimize soil health and biological functioning in diversified cropping systems. In the current context of high fossil fuel prices, the use of crop rotation combined with sulfur bentonite organo-mineral fertilizer (SBO) offers a more sustainable alternative to synthetic fertilizers such as NPK. With conventional fertilizers priced at €616/ton for nitrogen, €525/ton for phosphate, and €534/ton for potassium, SBO, priced at only €230/ton [7], provides a cost-effective and environmentally friendly option, particularly when considering its additional value in land restoration. This integrated approach supports both soil health and long-term economic viability for farmers.

5. Conclusions

The outcomes of this study demonstrate that both fertilization strategy and crop identity play pivotal roles in shaping soil chemical and biochemical properties across successive rotation cycles. Organic amendments, such as SBO and HM, significantly enhanced microbial activity, soil organic matter content, and cation exchange capacity. However, these benefits were accompanied by increased acidification and accelerated nutrient turnover—particularly when applied in conjunction with pepper cultivation, a biologically active crop. By contrast, the NPK fertilization promoted short-term nutrient availability but did not contribute substantially to long-term indicators of soil health.

These findings underscore the importance of considering the interaction between fertilizer type and crop species in the design of crop rotation systems. Organic inputs,

when strategically paired with microbial-stimulating crops, like pepper, may optimize soil microbial functioning and nutrient cycling. However, this approach also presents challenges, such as the need for careful monitoring of soil pH, nitrogen availability and synchronization, and the potential buildup of salinity.

Looking forward, the key challenge will be to refine and implement fertilization regimes that are not only crop-specific but responsive to site conditions and long-term sustainability goals. Future research should focus on developing dynamic nutrient management frameworks that integrate organic and inorganic inputs, account for microbial functional diversity, and mitigate the risks associated with acidification and nutrient imbalance. Moreover, there is a pressing need to evaluate these systems under varying climatic and soil conditions to ensure their adaptability and resilience.

In summary, our findings indicate that, under the specific pedoclimatic conditions of this study, organic fertilization can independently enhance soil ecosystem functioning, regardless of the crop rotation scheme. This suggests that neither crop rotation nor organic amendment alone can be universally regarded as superior; rather, their effectiveness is highly context-dependent. A synergistic strategy that combines tailored organic inputs with carefully planned crop rotations appears to be the most effective approach for improving soil health, strengthening agroecosystem resilience, and advancing sustainable agricultural productivity.

Author Contributions: Conceptualization, A.M. (Adele Muscolo); methodology, F.M.; software, A.M. (Angela Maffia); validation, A.M. (Angela Maffia); S.B., F.M., M.O., and C.M.; investigation, S.B.; resources, A.M. (Adele Muscolo); data curation, A.M. (Angela Maffia); writing—original draft preparation, A.M. (Adele Muscolo); writing—review and editing, A.M. (Angela Maffia) and A.M. (Adele Muscolo); visualization, S.B.; supervision, A.M (Adele Muscolo); project administration, A.M. (Adele Muscolo); funding acquisition, A.M. (Adele Muscolo). All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the project LIFE20 ENV/IT/000229—LIFE RecOrgFert PLUS, and funded by the LIFE Programme of the European Commission.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cole, M.B.; Augustin, M.A.; Robertson, M.J.; Manners, J.M. The Science of Food Security. *Npj Sci. Food* **2018**, *2*, 14. [CrossRef] [PubMed]
2. Techen, A.-K.; Helming, K. Pressures on Soil Functions from Soil Management in Germany. A Foresight Review. *Agron. Sustain. Dev.* **2017**, *37*, 64. [CrossRef]
3. Virto, I.; Imaz, M.; Fernández-Ugalde, O.; Gartzia-Bengoetxea, N.; Enrique, A.; Bescansa, P. Soil Degradation and Soil Quality in Western Europe: Current Situation and Future Perspectives. *Sustainability* **2014**, *7*, 313–365. [CrossRef]
4. Chew, K.W.; Chia, S.R.; Yen, H.-W.; Nomanbhay, S.; Ho, Y.-C.; Show, P.L. Transformation of Biomass Waste into Sustainable Organic Fertilizers. *Sustainability* **2019**, *11*, 2266. [CrossRef]
5. Muscolo, A.; Mauriello, F.; Marra, F.; Calabrò, P.S.; Russo, M.; Ciriminna, R.; Pagliaro, M. AnchoisFert: A New Organic Fertilizer from Fish Processing Waste for Sustainable Agriculture. *Glob. Chall.* **2022**, *6*. [CrossRef]
6. Maffia, A.; Marra, F.; Celano, G.; Oliva, M.; Mallamaci, C.; Hussain, M.I.; Muscolo, A. Exploring the Potential and Obstacles of Agro-Industrial Waste-Based Fertilizers. *Land* **2024**, *13*, 1166. [CrossRef]
7. Panuccio, M.R.; Marra, F.; Maffia, A.; Mallamaci, C.; Muscolo, A. Recycling of Agricultural (Orange and Olive) Bio-Wastes into Ecofriendly Fertilizers for Improving Soil and Garlic Quality. *Resour. Conserv. Recycl. Adv.* **2022**, *15*, 200083. [CrossRef]
8. Rodale Institute. Regenerative Organic Agriculture and Climate Change a Down- to-Earth Solution to Global Warming. 2014. Available online: https://rodaleinstitute.org/wp-content/uploads/rodale-white-paper.pdf (accessed on 2 March 2025).
9. Liu, C.; Plaza-Bonilla, D.; Coulter, J.A.; Kutcher, H.R.; Beckie, H.J.; Wang, L.; Floc'h, J.-B.; Hamel, C.; Siddique, K.H.M.; Li, L.; et al. Diversifying Crop Rotations Enhances Agroecosystem Services and Resilience. *Adv. Agron.* **2022**, *173*, 299–335. [CrossRef]

10. Pradhan, G.; Meena, R.S.; Kumar, S.; Lal, R. Utilizing Industrial Wastes as Compost in Wheat-Rice Production to Improve the above and below-Ground Ecosystem Services. *Agric. Ecosyst. Environ.* **2023**, *358*, 108704. [CrossRef]
11. Rai, A.; Singh, A.K.; Mishra, R.; Shahi, B.; Rai, V.K.; Kumari, N.; Kumar, V.; Gangwar, A.; Sharma, R.B.; Rajput, J.; et al. Sulphur in Soils and Plants: An Overview. *Int. Res. J. Pure Appl. Chem.* **2020**, *21*, 66–70. [CrossRef]
12. Lisowska, A.; Filipek-Mazur, B.; Kalisz, A.; Gorczyca, O.; Kowalczyk, A. Changes in Soil Sulfate Sulfur Content as an Effect of Fertilizer Granules Containing Elemental Sulfur, Halloysite and Phosphate Rock. *Agronomy* **2023**, *13*, 1410. [CrossRef]
13. Sayara, T.; Basheer-Salimia, R.; Hawamde, F.; Sánchez, A. Recycling of Organic Wastes through Composting: Process Performance and Compost Application in Agriculture. *Agronomy* **2020**, *10*, 1838. [CrossRef]
14. Maffia, A.; Scotti, R.; Wood, T.; Muscolo, A.; Lepore, A.; Acocella, E.; Celano, G. Transforming Agricultural and Sulfur Waste into Fertilizer: Assessing the Short-Term Effects on Microbial Biodiversity via a Metagenomic Approach. *Life* **2024**, *14*, 1633. [CrossRef] [PubMed]
15. Maffia, A.; Marra, F.; Canino, F.; Oliva, M.; Mallamaci, C.; Celano, G.; Muscolo, A. Comparative Study of Fertilizers in Tomato-Grown Soils: Soil Quality, Sustainability, and Carbon/Water Footprints. *Soil Syst.* **2023**, *7*, 109. [CrossRef]
16. Russo, M.; Di Sanzo, R.; Marra, F.; Carabetta, S.; Maffia, A.; Mallamaci, C.; Muscolo, A. Waste-Derived Fertilizer Acts as Biostimulant, Boosting Tomato Quality and Aroma. *Agronomy* **2023**, *13*, 2854. [CrossRef]
17. Marra, F.; Maffia, A.; Canino, F.; Greco, C.; Mallamaci, C.; Adele, M. Effects of fertilizer produced from agro-industrial wastes on the quality of two different soils. *Arch. Agron. Soil Sci.* **2023**, *69*, 3600–3618. [CrossRef]
18. Muscolo, A.; Mallamaci, C.; Settineri, G.; Calamara, G. Increasing soil and crop productivity by using agricultural wastes pelletized with elemental sulfur and bentonite. *Agron. J.* **2017**, *109*, 1900–1910. [CrossRef]
19. Muscolo, A.; Papalia, T.; Settineri, G.; Romeo, F.; Mallamaci, C. Three different methods for turning olive pomace in resource: Benefits of the end products for agricultural purpose. *Sci. Total Environ.* **2019**, *662*, 1–7. [CrossRef]
20. Muscolo, A.; Romeo, F.; Marra, F.; Mallamaci, C. Transforming agricultural, municipal and industrial pollutant wastes into fertilizers for a sustainable healthy food production. *J. Environ. Manag.* **2021**, *17*, 113771. [CrossRef]
21. Ben Said, I.; Muscolo, A.; Mezghani, I.; Chaieb, M. Reclaimed municipal wastewater for forage production. *Water Sci. Technol.* **2017**, *75*, 1784–1793.
22. Severson, R.C.; Shacklette, H.T. *Essential Elements and Soil Amendments for Plants: Sources and Use for Agriculture, 1017*; US Government Printing Office: Washington, DC, USA, 1988.
23. Bouyoucos, G.J. Hydrometer Method Improved for Making Particle Size Analyses of Soils[1]. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
24. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
25. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoff in organishen Kopern. *Anal. Chem.* **1883**, *22*, 354–358.
26. Kaminsky, R.; Müller, W.H. A recommendation against the use of alkaline soil extractions in the study of allelopathy. *Plant Soil* **1978**, *49*, 641–645. [CrossRef]
27. Box, J.D. Investigation of the Folin–Ciocalteau reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
28. Hendershot, W.H.; Duquette, M. A Simple Barium Chloride Method for Determining Cation Exchange Capacity and Exchangeable Cations. *Soil Sci. Soc. Am. J.* **1986**, *50*, 605–608. [CrossRef]
29. Maffia, A.; Marra, F.; Canino, F.; Battaglia, S.; Mallamaci, C.; Oliva, M.; Muscolo, A. Humic Substances from Waste-Based Fertilizers for Improved Soil Fertility. *Agronomy* **2024**, *14*, 2657. [CrossRef]
30. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
31. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951. [CrossRef]
32. Von Mersi, W.; Schinner, F. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
33. Sidari, M.; Santonoceto, C.; Anastasi, U.; Preiti, G.; Muscolo, A. Variations in four genotypes of lentil under NaCl-salinity stress. *Am. J. Agron. Biol. Sci.* **2008**, *3*, 410–416. [CrossRef]
34. Liu, Q.; Zhao, Y.; Li, T.; Chen, L.; Chen, Y.; Sui, P. Changes in Soil Microbial Biomass, Diversity, and Activity with Crop Rotation in Cropping Systems: A Global Synthesis. *Appl. Soil Ecol.* **2023**, *186*, 104815. [CrossRef]
35. Venter, Z.S.; Jacobs, K.; Hawkins, H.-J. The impact of crop rotation on soil microbial diversity: A meta-analysis. *Pedobiologia* **2016**, *59*, 215–223. [CrossRef]
36. Wang, L.; Zhao, Y.; Al-Kaisi, M.; Yang, J.; Chen, Y.; Sui, P. Effects of seven diversified crop rotations on selected soil health indicators and wheat productivity. *Agronomy* **2020**, *10*, 235. [CrossRef]

37. Zani, C.F.; Manning, D.A.C.; Abbott, G.D.; Taylor, J.A.; Cooper, J.; Lopez-Capel, E. Diversified Crop Rotations and Organic Amendments as Strategies for Increasing Soil Carbon Storage and Stabilisation in UK Arable Systems. *Front. Environ. Sci.* **2023**, *11*, 1113026. [CrossRef]
38. Zhou, J.; Xu, H.; Zhang, M.; Feng, R.; Xiao, H.; Xue, C. Impact of Organic Amendments on Black Wheat Yield, Grain Quality, and Soil Biochemical Properties. *Agronomy* **2025**, *15*, 961. [CrossRef]
39. Loaiza Puerta, V.; Pujol Pereira, E.I.; Wittwer, R.; van der Heijden, M.; Six, J. Improvement of Soil Structure through Organic Crop Management, Conservation Tillage and Grass-Clover Ley. *Soil Tillage Res.* **2018**, *180*, 1–9. [CrossRef]
40. Chen, Q.; Song, Y.; An, Y.; Lu, Y.; Zhong, G. Soil Microorganisms: Their Role in Enhancing Crop Nutrition and Health. *Diversity* **2024**, *16*, 734. [CrossRef]
41. Howe, J.A.; McDonald, M.D.; Burke, J.; Robertson, I.; Coker, H.; Gentry, T.J.; Lewis, K.L. Influence of Fertilizer and Manure Inputs on Soil Health: A Review. *Soil Secur.* **2024**, *16*, 100155. [CrossRef]
42. Souri, B.; Sayadi, Z. Efficiency of Sulfur-Bentonite Granules to Improve Uptake of Nutrient Elements by the Crop Plant Cultivated in Calcareous Soil. *Commun. Soil Sci. Plant Anal.* **2021**, *52*, 2414–2430. [CrossRef]
43. Huang, Y.; Kang, R.; Mulder, J.; Zhang, T.; Duan, L. Nitrogen Saturation, Soil Acidification, and Ecological Effects in a Subtropical Pine Forest on Acid Soil in Southwest China. *J. Geophys. Res. Biogeosci.* **2015**, *120*, 2457–2472. [CrossRef]
44. Tejada, M.; Garcia, C.; Gonzalez, J.L.; Hernandez, M.T. Use of Organic Amendment as a Strategy for Saline Soil Remediation: Influence on the Physical, Chemical and Biological Properties of Soil. *Soil Biol. Biochem.* **2006**, *38*, 1413–1421. [CrossRef]
45. Lal, R. Soil Carbon Sequestration to Mitigate Climate Change. *Geoderma* **2004**, *123*, 1–22. [CrossRef]
46. Titirmare, N.S.; Ranshur, N.J.; Patil, A.H.; Patil, S.R.; Margal, P.B. Effect of Inorganic Fertilizers and Organic Manures on Physical Properties of Soil: A Review. *Int. J. Plant Soil Sci.* **2023**, *35*, 1015–1023. [CrossRef]
47. Gao, Y.; Sun, S.; Xing, F.; Mu, X.; Bai, Y. Nitrogen Addition Interacted with Salinity-Alkalinity to Modify Plant Diversity, Microbial PLFAs and Soil Coupled Elements: A 5-Year Experiment. *Appl. Soil Ecol.* **2019**, *137*, 78–86. [CrossRef]
48. Badri, D.V.; Vivanco, J.M. Regulation and Function of Root Exudates. *Plant Cell Environ.* **2009**, *32*, 666–681. [CrossRef]
49. Maeder, P.; Fliessbach, A.; Dubois, D.; Gunst, L.; Fried, P.; Niggli, U. Soil Fertility and Biodiversity in Organic Farming. *Science* **2002**, *296*, 1694–1697. [CrossRef]
50. Philippot, L.; Raaijmakers, J.M.; Lemanceau, P.; van der Putten, W.H. Going Back to the Roots: The Microbial Ecology of the Rhizosphere. *Nat. Rev. Microbiol.* **2013**, *11*, 789–799. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Impacts of Conventional and Agri-Food Waste-Derived Fertilizers on Durum Wheat Yield, Grain Quality, and Soil Health: A Two-Year Field Study in Greece and Southern Italy

Adele Muscolo [1*], Kostantinos Zoukidis [2], Evangelous Vergos [2], Federica Alessia Marra [1], Ludovica Santoro [1], Mariateresa Oliva [1], Santo Battaglia [1], Angela Maffia [1] and Carmelo Mallamaci [1]

[1] Affiliation Agriculture Department, Mediterranea University, 89124 Reggio Calabria, Italy; [2]American Farm School of Thessaloniki, 570 01 Thermi, Greece; kzouki@afs.edu.gr (K.Z.); vvergos@afs.edu.gr (E.V.)

*Correspondence: amuscolo@unirc.it; Tel.: +39-0965169436

Applied Sciences

https://doi.org/10.3390/app151810292 v

Received: 11 August 2025

Revised: 15 September 2025

Accepted: 16 September 2025

Published: 22 September 2025

applied sciences

MDPI

Article

Impacts of Conventional and Agri-Food Waste-Derived Fertilizers on Durum Wheat Yield, Grain Quality, and Soil Health: A Two-Year Field Study in Greece and Southern Italy

Adele Muscolo [1,*], Kostantinos Zoukidis [2], Evangelous Vergos [2], Federica Alessia Marra [1], Ludovica Santoro [1], Mariateresa Oliva [1], Santo Battaglia [1], Angela Maffia [1] and Carmelo Mallamaci [1]

[1] Affiliation Agriculture Department, Mediterranea University, 89124 Reggio Calabria, Italy; federica.marra@unirc.it (F.A.M.); ludovica.santoro@unirc.it (L.S.); mariateresa.oliva@unirc.it (M.O.); santo.battaglia@unirc.it (S.B.); angela.maffia@unirc.it (A.M.); carmelo.mallamaci@unirc.it (C.M.)

[2] American Farm School of Thessaloniki, 570 01 Thermi, Greece; kzouki@afs.edu.gr (K.Z.); vvergos@afs.edu.gr (E.V.)

* Correspondence: amuscolo@unirc.it; Tel.: +39-09651694364

Abstract

Sustainable fertilization strategies are critical under climate change and the European Green Deal, particularly for Mediterranean cereal systems. Organic fertilizers derived from agro-industrial residues offer promising alternatives to conventional mineral inputs. This study evaluated RecOrgFert, a novel fertilizer composed of sulfur–bentonite and citrus-processing residues, in comparison with NPK (15-15-15) and horse manure across two years in Central Macedonia (Greece) and Apulia (Italy). Using a randomized complete block design, soil chemical and biological properties, plant growth, yield, and grain quality were assessed. RecOrgFert outperformed conventional fertilizers by enhancing soil fertility—raising organic matter 25–27% above control and further increasing it from 2023 to 2024 (up to +75% in Italy, +38% in Greece)—while improving cation exchange capacity, enzymatic activity, and soil water content. Wheat grown with RecOrgFert showed higher protein (up to 15.2%), antioxidant activity (DPPH > 37%, $ABTS^{+}$ > 26%), and phenolic and flavonoid content, with yields comparable to NPK. The unique sulfur and orange-residue composition distinguish RecOrgFert from standard fertilizers, promoting nutrient cycling, microbial activity, and bioactive compound accumulation. It represents a novel, circular, and climate-smart solution aligned with EU sustainability and circular economy objectives.

Keywords: organic fertilizer; soil fertility; wheat quality; agri-food waste recycling; sustainable agriculture

check for updates

Academic Editors: Marta Mesías and Tiane Finimundy

Received: 11 August 2025
Revised: 15 September 2025
Accepted: 16 September 2025
Published: 22 September 2025

Citation: Muscolo, A.; Zoukidis, K.; Vergos, E.; Marra, F.A.; Santoro, L.; Oliva, M.; Battaglia, S.; Maffia, A.; Mallamaci, C. Impacts of Conventional and Agri-Food Waste-Derived Fertilizers on Durum Wheat Yield, Grain Quality, and Soil Health: A Two-Year Field Study in Greece and Southern Italy. *Appl. Sci.* **2025**, *15*, 10292. https://doi.org/10.3390/app151810292

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated cereal crops globally and serves as a staple food for a large portion of the world's population, providing approximately 20% of the daily caloric and protein intake [1]. To sustain the productivity required for global food security, conventional agricultural systems have long relied on synthetic nitrogen (N), phosphorus (P), and potassium (K) fertilizers. While these inputs are effective in increasing yields, their prolonged and excessive use is associated with significant environmental challenges, including soil acidification, nutrient leaching, loss of biodiversity, and elevated greenhouse gas emissions [2–4]

In the context of climate change and resource scarcity, the transition to sustainable fertilization practices has become a key priority in global and European agricultural policy. Organic fertilizers derived from agri-food industrial wastes are gaining attention as viable alternatives that can recycle nutrients, reduce wastes, and improve soil health [5–7]. These materials contribute to increase organic matter and nutrients which in turn enhance soil physical structure, microbial activity, and nutrient cycling processes, ultimately benefiting crop performance and sustainability [7,8]. Beyond agriculture, the industrial sector also generates waste materials that can be repurposed into value-added products, contributing to waste valorization efforts. One example is sulfur, a byproduct recovered from crude oil refining. This recovered sulfur holds potential for agricultural applications, especially in light of research indicating that sulfur deficiency can impair nitrogen uptake efficiency and result in crop proteins with reduced levels of sulfur-containing amino acids, such as methionine—an essential component influencing the nutritional quality of crops. The use of waste-derived fertilizers perfectly aligns with the principles of the circular economy, which promotes resource efficiency, waste minimization, and carbon footprint reduction across all sectors, including agriculture [9]. In this regard, the European Green Deal and the Circular Economy Action Plan strongly encourage the agricultural reuse of organic wastes, which can also help address the issue of declining soil organic matter in Mediterranean agroecosystems [10,11].

Despite their potential, the agronomic performance of waste-derived fertilizers can vary significantly depending on factors such as feedstock composition, treatment process, soil type, and climatic conditions. As a result, field-based studies are essential to validate their efficacy under different agro-environmental contexts [8]. Furthermore, while several studies assessed either yield or soil health separately, fewer have integrated both agronomic, soil quality, yield and wheat quality across multiple years and more geographical locations [4,5].

The Mediterranean region, characterized by its climate variability, limited water resources, and declining soil fertility, provides a relevant setting for evaluating the sustainability of fertilization practices. Greece and Southern Italy (Apulia), in particular, are important wheat-producing regions where alternative fertilization strategies could contribute to climate-resilient and environmentally sound cereal production systems [12]. The present study aimed to evaluate the impact of conventional mineral nitrogen, phosphorous and potassium (NPK, 15-15-15) or organic horse manure (HM) fertilizers and RecOrgFert, fertilizer derived from industrial and agri-food wastes specifically orange waste residue of agro-food industry and sulfur as residue of oil industry on wheat yield, grain quality, and soil health. Conducted over two consecutive years in contrasting Mediterranean environments—Apulia (Southern Italy) and Central Macedonia (Northern Greece)—this study seeks to: (1) compare the agronomic effectiveness of different fertilizer treatments; (2) assess their effects on soil chemical and biological properties; and (3) analyze yield and quality of wheat comparing the consistence of treatment responses across sites. By integrating multiple indicators of performance, this research provides practical insights to guide sustainable nutrient management and policy development in Mediterranean cropping systems [6,11].

2. Materials and Methods

2.1. Study Sites and Experimental Design

Field experiments were conducted over two consecutive cropping seasons (2023–2024) in two Mediterranean locations: Southern Italy (Apulia) in 17 hectares in Cambisol (Eutric, Haplic) with a sandy-loam texture (Sand, 37.2%; Silt, 49.9% and Clay 12.9%) and Central Macedonia Northern Greece (Thessaloniki) 12 hectares in Cambisol (Eutric, Calcaric, Clayic)

with clay-loam texture (Sand 38%; Silt 30% and Clay 32%), classified in accordance with FAO Word Reference Base (WRB). Both sites are representative of typical durum wheat (*Triticum durum* Desf.) cultivation areas in Mediterranean Environment but differ in soil types, climatic conditions, and agricultural history (Supplementary Material Figure S1. (a) Layout of the 17-hectare experimental field in Apulia, showing the distribution of the different treatments; (b) layout of the 12-hectare experimental field in Greece, showing the distribution of the different treatments, Tables S1–S3). Greece Thessaloniki (2023–2024) experienced a mild but stormy winter, a moderate spring, an exceptionally hot and dry summer, and a wet autumn with occasional severe storms (Supplementary Material Table S1). Southern Italy (Apulia) (2023–2024) experienced mild rainy winters, moderate springs, extremely hot and dry summers, and wetter autumns, typical of the Mediterranean inland climate (Supplementary Material Table S2). Basic management practices were the same in both locations (Greece and Italy): crop rotation with oat before wheat sowing; soil management including plowing, harrowing, and sowing with a precision seeder; and no phytosanitary treatments were applied.

In each location, a randomized complete block design (RCBD) was implemented with three replicates per treatment. The experimental treatments included: control (CTR)—No fertilized soil that represents the basic physical and chemical soil parameters; chemical fertilizer NPK (15-15-15); organic fertilizer (horse manure, HM); and sulfur bentonite plus orange wastes (RecOrgFert). RecOrgFert was produced by Steel Belt System s.r.l, in pellets of 3/4 mm diam. The mixture was formed by 85% of liquid sulfur, 10% of bentonite clay (as support and carrier), and 5% of fresh orange waste pastilled in a special belt system apparatus. Synthetic fertilizer NPK (15-15-15) containing 150 g/kg of N, 150 g/kg P_2O_5 and 150 g/kg of K_2O; HM containing 20 g/kg of N, 20 g/kg of P_2O_5, and 15 g/kg of K_2O. RecOrgFert composed by sulfur (85%)–bentonite (10%) + orange residue (5%), containing 15 g/Kg of N, 49 g/Kg of K and 1.09 g/Kg of P_2O_5.

Fertilizers were applied pre-sowing in the second half of December and at the vegetative state (25 cm height) at the end of February, at rates standardized to equivalent nitrogen content (150 kg N ha^{-1}), following local agronomic guidelines. At the Apulia site, the total experimental area of 17 hectares was divided into four main plots of approximately 4 hectares each, assigned as follows: control, NPK, RecOrgFert and horse manure. Each plot measured roughly 1335 m in length and 90 m in width, ensuring uniformity, particularly with respect to altitude, which ranged from 147 m to 203 m. Each main plot was further subdivided into three 1.33-hectare subplots, which served as replicates for the experimental treatments. At the Thessaloniki site, the 12-hectare area was similarly divided into four main plots of 3.0 hectares each, maintaining homogeneity among plots (Figure S1a,b). Each main plot was further subdivided into three 1-hectare subplots, which served as replicates for the experimental treatments.

2.2. Soil Analysis

Soil samples were collected from the upper 0–35 cm layer of each plot at three sampling times: (i) before the start of the experiment (T0, baseline), (ii) immediately after harvest in 2023, and (iii) immediately after harvest in 2024. For each plot, five subsamples were taken and combined into a composite sample for analysis. The choice of a 0–35 cm sampling depth reflects the active root zone of durum wheat, where nutrient uptake occurs predominantly, thus making this layer representative for assessing comparative soil conditions relevant to wheat growth and nutrient availability. Therefore, this sampling depth was considered representative for assessing comparative soil conditions relevant to wheat growth and nutrient availability. For each plot, five subsamples were taken, combined into a composite sample, and analyzed for various soil properties

The initial soil characteristics determined at time zero are reported in Table S3 of the Supplementary Materials. Results of soil analyses after harvest are presented in Tables 1 and 2 of the main text.

Table 1. Chemical and biochemical properties of the soil in the experiment in Greece.

	CTR	NPK	HM	RecOrgFert
		2023		
pH	8.0 ^{a}A ± 0.1	7.9 ^{b}A ± 0.1	7.9 ^{b}A ± 0.1	7.9 ^{b}A ± 0.1
OC	2.32 ^{c}A ± 0.15	4.00 ^{a}A ± 0.20	3.44 ^{b}A ± 0.18	2.34 ^{c}B ± 0.15
OM	4.0 ^{c}A ± 0.25	6.90 ^{a}A ± 0.30	5.93 ^{b}A ± 0.28	4.03 ^{c}B ± 0.25
C/N	19.33 ^{b}A ± 1.2	25.00 ^{a}B ± 1.5	22.93 ^{a}A ± 1.4	18.00 ^{b}B ± 1.0
CEC	12.73 ^{b}B ± 1.0	10.72 ^{b}B ± 1.1	13.60 ^{a}B ± 1.2	10.71 ^{b}B ± 1.0
Tot N	0.12 ^{b}B ± 0.01	0.16 ^{a}A± 0.01	0.15 ^{a}A ± 0.01	0.13 ^{b}A ± 0.01
EC	0.37 ^{b}B ± 0.03	0.40 ^{b}B ± 0.02	0.40 ^{b}B ± 0.02	0.43 ^{a}A ± 0.02
WC	13.10 ^{ab}A ± 1.5	11.10 ^{b}A ± 1.3	12.00 ^{ab}A ± 1.4	16.40 ^{a}A ± 1.8
		2024		
pH	8.0 ^{a}A ± 0.1	7.9 ^{b}A ± 0.1	7.7 ^{c}A ± 0.1	7.6 ^{c}B ± 0.1
OC	2.5 ^{c}A ± 0.16	4.10 ^{a}A ± 0.20	3.0 ^{b}B ± 0.16	3.2 ^{b}A ± 0.18
OM	4.4 ^{c}A ± 0.28	7.1 ^{a}A ± 0.35	5.2 ^{b}B ± 0.28	5.5 ^{b}A ± 0.30
C/N	14.2 ^{c}B ± 1.2	29.3 ^{a}A ± 1.6	23.4 ^{b}A ± 1.3	29.0 ^{a}A ± 1.5
CEC	22.8 ^{c}A ± 2.0	23.4 ^{bc}A ± 2.1	28.9 ^{a}A ± 2.3	24.1 ^{ab}A ± 2.2
Tot N	0.18 ^{a}A ± 0.02	0.14 ^{b}A ± 0.01	0.13 ^{b}A ± 0.01	0.11 ^{c}A ± 0.01
EC	0.82 ^{a}A ± 0.05	0.50 ^{b}A ± 0.03	0.57 ^{b}A ± 0.03	0.44 ^{b}A ± 0.02
WC	13.9 ^{b}A ± 1.5	12.0 ^{b}A ± 1.4	14.2 ^{ab}A ± 1.5	14.9 ^{a}A ± 1.6

CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert = bentonite sulfur + orange pomace. pH; OC = organic carbon (%); OM = organic matter (%); C/N = carbon–nitrogen ratio; CEC = cation exchange capacity (meq/100 g); Tot N = total nitrogen (%); EC = electrical conductivity (dS/m); WC = soil moisture (%). Data are the means of three replicates ± standard deviation. Different lowercase letters in the same row indicate significant differences (Tukey's test. $p \leq 0.05$). Different uppercase letters in the same column indicate significant differences (Tukey's test. $p \leq 0.05$) for the same parameters between the different years.

Table 2. Plant growth and grain biochemical parameters in the experiment in Greece (2023 and 2024).

	CTR	NPK	HM	RecOrgFert
		2023		
Plant height	176.0 ^{b}A ± 4.5	202.0 ^{a}A ± 5.0	203.0 ^{a}A ± 4.8	181.0 ^{ab}A ± 4.3
Seed/ear	35 ^{b}A ± 3	41 ^{a}B ± 3	37 ^{b}A ± 3	42 ^{a}B ± 2
Yield	0.177 ^{ab}A ± 0.018	0.198 ^{a}A ± 0.015	0.175 ^{ab}A ± 0.017	0.157 ^{b}A ± 0.016
Dry Seed	0.7 ^{b}A ± 0.1	1.0 ^{ab}A ± 0.1	2.2 ^{a}A ± 0.2	1.1 ^{ab}A ± 0.1
Proteins	12.0 ^{b}A ± 0.8	12.3 ^{ab}B ± 0.9	10.5 ^{c}B ± 0.8	15.1 ^{a}A ± 1.0
Dry gluten	14.1 ^{b}B ± 1.0	21.6 ^{a}A ± 1.2	18.9 ^{a}A ± 1.1	20.0 ^{a}B ± 1.1
β-carotene	0.50 ^{b} B ± 0.05	0.61 ^{ab}B ± 0.06	0.66 ^{a}B ± 0.07	0.62 ^{ab}B ± 0.06
TP	19.87 ^{c}A ± 0.85	21.76 ^{b}A ± 0.92	22.31 ^{ab}A ± 0.97	23.02 ^{a}A ± 1.04
TF	10.02 ^{c}B ± 0.47	18.89 ^{a} B ± 0.76	16.94 ^{b}A ± 0.73	17.62 ^{a}B ± 0.81
DPPH	32.85 ^{b}A ± 1.22	35.01 ^{ab}A ± 1.35	35.89 ^{a}A ± 1.33	36.45 ^{a}A ± 1.41
$ABTS^{+}$	23.67 ^{c}B ± 1.03	24.02 ^{c}A ± 1.18	24.88 ^{b}B ± 1.15	25.91 ^{a}A ± 1.26
		2024		
Plant height	161.0 ^{c}B ± 4.0	190.2 ^{a}B ± 4.5	186.8 ^{a}B ± 4.2	173.0 ^{b}A ± 4.1
Seed/ear	36 ^{b}A ± 3	53 ^{a}A ± 4	36 ^{b}A ± 3	50 ^{a}A ± 3
Yield	0.165 ^{ab}A ± 0.06	0.175 ^{a}B ± 0.07	0.139 ^{b}B ± 0.013	0.152 ^{a}A ± 0.05
Dry Seed	0.5 ^{ab}A ± 0.1	0.4 ^{b} B ± 0.1	1.1 ^{a}B ± 0.1	0.6 ^{ab}B ± 0.1
Proteins	12.8 ^{b}A ± 0.9	14.7 ^{ab}A ± 1.1	14.9 ^{a}A ± 1.0	16.6 ^{a}A ± 1.2
Dry gluten	18.0 ^{b}A ± 1.2	20.0 ^{a}A ± 1.1	17.8 ^{b}A ± 1.0	23.4 ^{a}A ± 1.3

Table 2. *Cont.*

	CTR	NPK	HM	RecOrgFert
β-carotene	2.20 ^{b}A ± 0.20	2.32 ^{ab}A ± 0.21	2.35 ^{a}A ± 0.22	2.29 ^{ab}A ± 0.20
TP	12.03 ^{b}B ± 0.56	11.37 ^{b}B ± 0.61	20.92 ^{a}B ± 1.02	19.65 ^{a}B ± 0.94
TF	11.12 ^{c}A ± 0.49	19.91 ^{a}A ± 0.92	17.41 ^{b}A ± 0.87	22.74 ^{a}A ± 1.03
DPPH	15.88 ^{c}B ± 0.88	23.45 ^{b}B ± 1.21	29.87 ^{a}B ± 1.38	26.12 ^{ab}B ± 1.30
$ABTS^{+}$	25.33 ^{b}A ± 1.11	26.12 ^{ab}A ± 1.25	28.15 ^{a}A ± 1.33	27.03 ^{a}A ± 1.19

CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert = bentonite sulfur + orange pomace. Plant height (cm); Seed/ear; Yield (Kg/m^2); Dry Seed (g/plant); Proteins (%); Dry gluten (% s.s.); β-carotene (mg/100 g). TP = total phenols (mg GAE g^{-1}); TF = total flavonoids (mg QE g^{-1}); DPPH = 2.2-difenil-1-picrilidrazile (% inhibition); $ABTS^{+}$ = 2.2′-azino-bis-3-etilbenzotiazolin-6-solfonato (% inhibition). Data are the means of three replicates ± standard deviation. Different lowercase letters in the same row indicate significant differences (Tukey's test. $p \leq 0.05$). Different uppercase letters in the same column indicate significant differences (Tukey's test. $p \leq 0.05$) for the same parameters between the different years.

Electrical conductivity (EC) was measured in a 1:5 (soil–distilled water) suspension, shaken mechanically at 15 rpm for 1 h to dissolve soluble salts, and recorded using a Hanna conductivity meter. Soil pH was determined in a 1:2.5 soil-to-water suspension using a glass electrode. Organic carbon content was quantified via the dichromate oxidation method, as described by Walkley and Black [13]. Total nitrogen (TN) was determined using the Kjeldahl digestion technique [14], and the carbon-to-nitrogen (C/N) ratio was calculated accordingly. Water-soluble phenolic compounds were extracted following the procedures outlined by Kaminsky and Müller [15,16]. All analyses were performed following standardized protocols as outlined in ISO 10390 and ISO 14240 [17,18]. Soil classification followed the FAO World Reference Base (WRB) system.

2.3. Yield Measurements

At crop maturity, wheat plants were manually harvested from a 4 m^2 area in each plot to avoid edge effects. The following yield parameters were recorded: plant height (cm), Seed/ear; Yield (Kg/m^2), calculated on the basis of the total mass of grain harvested and corrected to 13% moisture content, following standard normalization procedures [19]. Dry seed weight (g/plant) was determined to assess seed quality while avoiding the influence of moisture. Grain samples were dried in an oven at a controlled temperature until they reached constant weight, and the dry weight was then measured in grams (g) [19].

2.4. Grain Quality Analysis

Grain quality in terms of protein and gluten content, beta-carotene, total phenols and total flavonoids, antioxidant capacity ($ABTS^{+}$ and DPPH) was assessed using a subsample of dried and cleaned kernels from each plot. Protein content (%) was measured by using near-infrared spectroscopy (NIRS), a widely adopted non-destructive method for evaluating grain quality [20]. Gluten content and index have been measured by standard wet chemistry (ICC method No. 137/1), which provides robust indicators of baking quality [21]. The β-carotene content was determined by extraction with a solvent mixture (acetone–ethanol–hexane, 1:1:2, *v*/*v*/*v*), followed by spectrophotometric quantification at 450 nm, according to Saini et al. [22]. Results were expressed in mg/100 g of dry weight. Grain moisture content was adjusted to 13% for standardization. Total phenolic content was quantified using the Folin–Ciocalteu method [23], as previously described by Velioglu et al. [24]. For total flavonoid content, a colorimetric assay based on complex formation with aluminum chloride was employed, following the protocol developed by Djeridane et al. [25]. Antioxidant capacity was assessed using two standard assays. The $ABTS^{+}$ radical cation decolorization assay was performed according to the method of Re et al. [26], while the DPPH free radical scavenging assay, utilizing 2,2-diphenyl-1-picrylhydrazyl,

followed the procedure outlined by Barreca et al. [27]. Analytical precision was verified using certified reference materials. All tests were conducted in triplicate.

2.5. Statistical Analysis

Analysis of variance (ANOVA) was performed for all data sets. One-way ANOVA followed by Tukey's HSD (honestly significant difference) test was used to assess the effects of fertilizers on the various measured parameters. Comparisons were conducted to assess the effects of fertilizers on each individual parameter in respect to control. To explore the relationships between soil properties and crop parameters, correlation analyses were performed using the changes (Δ) in soil properties and crop quality traits induced by the different fertilizers. For each soil parameter, Δ values (e.g., ΔpH, ΔOC, ΔOM, ΔC/N, etc.) were calculated as the difference between post-harvest values and the baseline values measured before treatment application (time zero). These Δ values were then correlated with the corresponding changes in crop parameters (e.g., ΔProteins, ΔDry gluten, Δβ-carotene). Pearson correlation matrix analysis was applied to these datasets.

All statistical analyses were performed using MATLAB (version R2024b, The MathWorks Inc., Natick, MA, USA). Effects were considered significant at $p \leq 0.05$.

3. Results

3.1. Experiments in Central Macedonia Greece

3.1.1. Soil Properties

Over the two-year period, all fertilization treatments induced significant changes in soil chemical parameters compared to the control (CTR), with distinct trends across 2023 and 2024. In 2023 (Table 1), the application of NPK significantly increased organic matter (OM) (6.9%) compared to the control (4.0% OM), indicating a rapid nutrient soil incorporation that improved water retention, soil structure and nutrient availability. Horse manure (HM) and RecOrgFert also enhanced these parameters, although to a lesser extent. By 2024 (Table 1), OM were the highest under NPK (7.1%) and RecOrgFert (5.5%), with RecOrgFert showing notable improvement from the previous year (+37.5%). Interestingly, the increment of organic matter in percentage over the two years was more in presence of RecOrgFert (+25%) than NPK (+2.8%). CEC increased across all treatments, particularly under HM (28.92 meq/100 g). Soil pH decreased slightly across treatments in 2024 compared to 2023, under RecOrgFert (from 7.9 to 7.6), due to the presence of sulfur that acidify the soil overtime. Electrical conductivity increased in 2024 compared to 2023 except for RecOrgFert suggesting higher soluble salt content.

3.1.2. Plant Growth and Yield

NPK and RecOrgFert treatments consistently improved plant height and seed set across both years. In 2023 (Table 2), plants grown with NPK and HM were the tallest (~202–203 cm), whereas RecOrgFert produced the highest seeds per ear (42). In 2024, overall plant height declined—likely due to climatic variability—yet NPK (190.2 cm), HM (186.8 cm), and RecOrgFert (173.0 cm) still outperformed the control. Seed/ear values increased in 2024, particularly under NPK (53) and RecOrgFert (50). Yield was highest under NPK in both years (0.198 Kg/m^2 in 2023; 0.175 Kg/m^2 in 2024), followed by RecOrgFert. Although HM promoted higher dry seed mass in 2023 (2.2 g/plant), this benefit diminished in 2024, while RecOrgFert maintained stable yields.

3.1.3. Grain Biochemical Quality

Protein and gluten contents were enhanced under RecOrgFert, reaching 16.6% and 23.4% in 2024, respectively, indicating improved nutritional quality. Interestingly, β-

carotene content nearly tripled across all treatments, rising from ~0.62 to >2.29 mg/100 g between years, suggesting strong environmental or varietal influences.

RecOrgFert and HM proved most effective in enhancing phenolic compounds and antioxidant capacity, though their relative performance varied across years. In 2023 (Table 2), RecOrgFert clearly dominated, showing the highest total phenols (23.02 mg GAE/g), strong $ABTS^+$ activity (25.91%), and the greatest DPPH scavenging capacity (36.45%), suggesting a robust induction of antioxidant pathways. By contrast, in 2024, the phenolic profile shifted: HM produced the highest total phenols (20.92 mg GAE/g), while RecOrgFert excelled in flavonoid accumulation (22.74 mg QE/100 g), indicating that different fertilizer strategies may favor distinct classes of phenolics. Antioxidant assays further reflected this shift—HM led in DPPH activity (29.87%), pointing to stronger radical scavenging, whereas both RecOrgFert and HM sustained high $ABTS^+$ activities (>27%), demonstrating consistently enhanced antioxidant potential relative to the control. These results highlight that while RecOrgFert stimulates a broad antioxidant response, HM appears particularly effective under variable conditions in maintaining phenolic content and specific antioxidant activities.

3.1.4. Soil and Grain Parameters: The Correlation

Regarding the results of Greece in the first year (2023), representing in Figure S2 (Supplementary Material), in the control (CTR), correlations revealed a predominantly negative pattern: pH, organic carbon (ΔOC), organic matter (ΔOM), and water content (ΔWC) were negatively correlated with ΔProteins, ΔDry gluten, Δβ-carotene, and antioxidant capacity. Conversely, electrical conductivity (ΔEC) showed strong positive correlations with nutritional parameters (ΔProteins, ΔDry gluten, and Δβ-carotene). The ΔC/N ratio and total nitrogen (ΔTotN) displayed more variable relationships, with positive values mainly associated with antioxidants. In the NPK treatment, ΔOM, ΔC/N ratio, ΔTotN, and ΔEC were strongly and positively correlated with ΔProteins, ΔDry gluten, and Δβ-carotene. By contrast, ΔpH and ΔWC were negatively correlated with most variables. Antioxidant capacity was primarily correlated with ΔC/N ratio, ΔTotN, and ΔEC. In the HM treatment, ΔOM was positively correlated with ΔProteins, ΔDry gluten, and Δβ-carotene, but negatively with total polyphenols (ΔTP) and flavonoids (ΔTF). Both ΔOC and ΔEC showed strong negative correlations with ΔProteins, ΔDry gluten, and Δβ-carotene. A similar trend was observed for ΔpH, which was negatively related to nutritional parameters but positively associated with antioxidant activity (particularly $\Delta ABTS^+$). Finally, ΔTotN and the ΔC/N ratio showed positive correlations with ΔTP and antioxidants. In the RecOrgFert treatment, ΔOC and ΔOM were positively correlated with nutritional parameters (ΔProteins, ΔDry gluten, and Δβ-carotene). Conversely, ΔpH was negatively correlated with nutrients but positively related to ΔTP and ΔDPPH. The ΔC/N ratio and cation exchange capacity (ΔCEC) were positively correlated with $\Delta ABTS^+$, while ΔTotN and ΔEC exhibited weak or variable correlations. ΔWC showed only weak positive relationships with nutritional traits. (Figure S2, Supplementary Materials).

Regarding the results from Greece in the second year (2024), the correlation matrix (Figure S3, Supplementary Materials) for the control (CTR) revealed clear trends. Strong positive correlations were observed between ΔOC, ΔOM, ΔC/N, ΔCEC, and ΔWC with ΔProteins and Δβ-carotene, whereas ΔTotN showed negative correlations with these traits. Organic-related variables (ΔOC, ΔOM, ΔC/N, ΔCEC, and ΔWC) were negatively associated with ΔTP and ΔTF but displayed positive associations with the antioxidant activities ΔDPPH and $\Delta ABTS^+$. In contrast, ΔpH exhibited negative correlations with most nutritional traits, particularly with $\Delta ABTS^+$ (Figure S3, Supplementary Materials).

In the NPK treatment, negative correlations emerged between ΔpH, ΔOC, ΔOM, and ΔC/N with ΔProteins and ΔTF. Regarding antioxidants, ΔDPPH was strongly and positively correlated with ΔpH, ΔOC, ΔOM, and ΔC/N, whereas $\Delta ABTS^+$ was negatively correlated with almost all variables.

In the HM treatment, ΔpH showed strong negative correlations with ΔProteins, ΔDry gluten, Δβ-carotene, ΔTP, and ΔDPPH, while ΔOC and ΔOM exhibited weak or variable associations. In contrast, ΔC/N, ΔCEC, ΔTotN, ΔEC, and ΔWC displayed strong positive correlations with nutritional components, with ΔEC and ΔWC being particularly closely related to ΔProteins and Δβ-carotene. With respect to antioxidants, ΔDPPH correlated positively with ΔC/N and ΔTotN, while $\Delta ABTS^+$ showed weak positive associations with ΔpH and ΔOM.

In the RecOrgFert treatment, ΔpH correlated strongly and positively with ΔProteins, ΔDry gluten, and Δβ-carotene, but negatively with ΔTP and ΔDPPH. On the other hand, ΔOC and ΔOM were positively correlated with antioxidants (ΔTF, ΔDPPH, and $\Delta ABTS^+$), but negatively with nutrients and Δβ-carotene. ΔCEC showed positive correlations with both nutrients and $\Delta ABTS^+$, whereas ΔTotN and ΔEC were negatively associated with ΔProteins, ΔDry gluten, Δβ-carotene, and $\Delta ABTS^+$.

Regarding the results of Italy in the first year (2023), representing in Figure S4 (Supplementary Material), in the control (CTR), positive correlations were observed between ΔOC, ΔOM, ΔTotN, and ΔEC with ΔProteins and ΔDry gluten, while ΔWC was negatively associated with these nutritional traits. Total polyphenols (ΔTP) and flavonoids (ΔTF) were negatively correlated with ΔpH and ΔCEC, whereas the antioxidants ΔDPPH and $\Delta ABTS^+$ showed positive correlations with ΔOC, ΔOM, and ΔC/N. Interestingly, ΔpH was positively correlated with Δβ-carotene but negatively with ΔTP and ΔTF.

In the NPK treatment, ΔOC and ΔOM were positively correlated with ΔProteins but negatively with ΔC/N. Nutritional variables (ΔProteins, ΔDry gluten, and Δβ-carotene) were mainly associated with ΔCEC and ΔWC. ΔTF showed a negative correlation with ΔEC, whereas ΔDPPH was positively associated with ΔOC, ΔOM, ΔCEC, and ΔWC. $\Delta ABTS^+$ appeared primarily correlated with ΔTotN, while showing negative correlations with ΔEC. In the HM treatment, strong negative correlations were detected between ΔpH and ΔC/N with Δβ-carotene and other nutritional traits. ΔOM was positively correlated with ΔDry gluten, while ΔOC was positively associated with ΔProteins and Δβ-carotene. With regard to antioxidants, ΔTP displayed negative correlations with ΔOM, ΔC/N, and ΔCEC, whereas $\Delta ABTS^+$ was positively related to ΔOC, ΔOM, and ΔWC. In the RecOrgFert treatment, strong positive correlations were observed between ΔOM and ΔEC with ΔProteins and ΔDry gluten, whereas ΔOC was negatively correlated with Δβ-carotene. ΔCEC and ΔWC showed weak positive associations with nutritional traits. Antioxidants presented a more complex pattern: ΔOC and ΔOM were strongly negatively correlated with $\Delta ABTS^+$ but positively associated with ΔTF and ΔDPPH; in addition, ΔCEC and ΔTotN were positively correlated with ΔDPPH. (Figure S4, Supplementary Material). Overall, this treatment displayed a mixed correlation profile, with ΔOM and ΔEC supporting nutritional traits, while ΔOC and ΔpH appeared to play a greater role in shaping antioxidant responses.

Regarding the results of Italy in the second year (2024), representing in Figure S5 (Supplementary Material), In the control (CTR), positive correlations were observed between ΔProteins, ΔpH, the ΔC/N ratio, and ΔTotN, whereas ΔDry gluten was mainly associated with organic matter (ΔOM). Δβ-carotene showed positive correlations with electrical conductivity (ΔEC), ΔC/N ratio, and ΔCEC, while antioxidant activity (ΔDPPH and $\Delta ABTS^+$) displayed contrasting patterns. In the NPK treatment, proteins were positively correlated with ΔCEC and ΔOM, while ΔDry gluten showed negative correlations with ΔpH, ΔOC, and ΔTotN. Δβ-carotene was positively associated with ΔOM and ΔTotN but negatively

with ΔC/N and ΔEC. Phenolic compounds (ΔTP and ΔTF) exhibited positive correlations with ΔpH, ΔOC, ΔOM, ΔWC, and ΔTotN, whereas antioxidant activity showed variable trends, being positively associated with ΔEC but negatively with ΔOC and ΔTotN. In the HM treatment, ΔProteins were negatively correlated with ΔpH, ΔC/N ratio, and ΔCEC, but positively with ΔTotN, ΔOM, and ΔOC. ΔDry gluten was positively associated with ΔTotN, while Δβ-carotene showed positive correlations with ΔC/N ratio and negative correlations with ΔOC and ΔOM. Antioxidant activities exhibited weaker and less consistent associations. The RecOrgFert treatment generated strong and consistent correlations. ΔProteins were positively related to ΔC/N ratio, ΔOM, and ΔEC, but negatively with ΔWC. ΔDry gluten was positively associated with ΔOC and ΔWC, whereas Δβ-carotene showed strong positive correlations with ΔpH. Phenolic compounds (ΔTP and ΔTF) were mainly negatively associated with ΔOM and ΔC/N ratio, while antioxidant activity ($\Delta ABTS^{+}$) displayed a markedly positive correlation with ΔpH (Figure S5, Supplementary Material).

3.2. Experiments in Italy (Apulia)

3.2.1. Soil Properties

In Apulia, soil properties responded distinctly to both organic and synthetic amendments. In 2023 (Table 3), the control plot exhibited the highest organic carbon (OC) content (1.24%), while NPK, HM, and RecOrgFert all showed comparably lower values (~0.94–0.97%). This pattern suggests limited short-term mineralization and incorporation of organic matter following the initial application of amendments.

Table 3. Chemical and biochemical properties of soil in the experiment in Italy (Apulia, 2023–2024).

	CTR	NPK	HM	RecOrgFert
		2023		
pH	7.60 ^{b}A ± 0.12	7.80 ^{a}A ± 0.09	7.70 ^{ab}A ± 0.08	7.80 ^{a}A ± 0.11
OC	1.24 ^{a}A ± 0.18	0.95 ^{b}B ± 0.22	0.94 ^{b}A ± 0.19	0.97 ^{b}B ± 0.26
OM	2.14 ^{a}A ± 0.31	1.64 ^{b}B ± 0.28	1.62 ^{b}A ± 0.24	1.67 ^{b}B ± 0.35
C/N	0.12 ^{a}A ± 0.015	0.12 ^{a}A ± 0.018	0.13 ^{a}B ± 0.020	0.09 ^{b}A ± 0.012
CEC	29.50 ^{a}A ± 3.20	27.90 ^{b}A ± 2.90	26.20 ^{b}A ± 3.40	19.30 ^{c}B ± 2.60
Tot N	0.15 ^{a}A ± 0.025	0.11 ^{b}A ± 0.032	0.12 ^{b}A ± 0.035	0.09 ^{c}B ± 0.028
EC	0.36 ^{a}B ± 0.08	0.34 ^{a}B ± 0.12	0.34 ^{a}B ± 0.11	0.33 ^{a}B ± 0.09
WC	12.93 ^{b}A ± 2.10	11.21 ^{b}A ± 1.95	12.29 ^{b}A ± 2.25	15.98 ^{a}A ± 2.80
		2024		
pH	7.80 ^{a}A ± 0.14	7.80 ^{a}A ± 0.10	7.80 ^{a}A ± 0.11	7.80 ^{a}A ± 0.13
OC	1.34 ^{c}A ± 0.31	1.87 ^{a}A ± 0.42	0.95 ^{d}A ± 0.24	1.64 ^{a}A ± 0.38
OM	2.31 ^{b}A ± 0.12	3.22 ^{a}A ± 0.58	1.64 ^{d}A ± 0.12	2.93 ^{a}A ± 0.41
C/N	0.14 ^{b}A ± 0.022	0.10 ^{c}A ± 0.016	0.20 ^{a}A ± 0.028	0.12 ^{b}A ± 0.019
CEC	29.50 ^{a}A ± 2.10	27.10 ^{a}A ± 1.60	16.50 ^{c}B ± 1.40	26.1 ^{a}A ± 1.80
Tot N	0.19 ^{a}A ± 0.045	0.18 ^{a}A ± 0.038	0.19 ^{a}A ± 0.042	0.20 ^{a}A ± 0.048
EC	0.71 ^{b}A ± 0.15	0.69 ^{b}A ± 0.13	0.70 ^{b}A ± 0.14	0.91 ^{a}A ± 0.18
WC	14.01 ^{a}A ± 1.40	12.12 ^{a}A ± 1.15	13.89 ^{a}A ± 2.50	13.97 ^{a}A ± 2.65

CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert = bentonite sulfur + orange pomace. pH; OC = organic carbon (%); OM = organic matter (%); C/N = carbon–nitrogen ratio; CEC = cation exchange capacity (meq/100 g); Tot N = total nitrogen (%); EC = electrical conductivity (dS/m); WC = soil moisture (%). Data are the means of three replicates ± standard deviation. Different lowercase letters in the same row indicate significant differences (Tukey's test. $p \leq 0.05$). Different uppercase letters in the same column indicate significant differences (Tukey's test. $p \leq 0.05$) for the same parameters between the different years.

By 2024, however, clear differences emerged. Both RecOrgFert and NPK significantly improved soil organic carbon (1.64% and 1.87%, respectively) and organic matter content (2.93% and 3.22%), indicating enhanced nutrient cycling and organic matter turnover relative to the previous year. In contrast, HM maintained lower values, suggesting reduced

efficiency in sustaining soil organic reserves. Cation exchange capacity (CEC) declined under HM (16.5 meq/100 g), which may reflect limited contributions to soil colloidal stability, whereas control and NPK plots preserved higher CEC values. Interestingly, electrical conductivity (EC) increased notably under RecOrgFert in 2024 (0.91 dS/m), likely reflecting greater ionic activity and nutrient availability in the soil solution.

Overall, these results highlight that RecOrgFert and NPK were more effective in improving soil organic status and nutrient-holding capacity over time, while HM showed weaker effects, particularly on CEC and organic matter maintenance. The elevated EC under RecOrgFert also points to an enriched soil nutrient environment, potentially contributing to improved crop performance observed in the same period.

3.2.2. Plant Growth and Yield

NPK and RecOrgFert consistently enhanced wheat growth and productivity. In 2023 (Table 4), plant height peaked with NPK (105 cm) and RecOrgFert (101 cm), compared to 78 cm in unfertilized soil. Yield followed similar trends, with NPK (0.32 Kg/m^2) and RecOrgFert (0.29 Kg/m^2) far outperforming the control (0.22 Kg/m^2).

Table 4. Plant growth and grain biochemical parameters in the experiment in Italy (Apulia, 2023 and 2024).

	CTR	NPK	HM	RecOrgFert
		2023		
Plant height	78.0 ^{c}A ± 4.50	105.00 ^{a}A ± 8.30	84.0 ^{b}A ± 4.20	101.0 ^{a}A ± 7
seed/ear	36.0 ^{b}B ± 4.20	43.0 ^{a}A ± 5.80	37.0 ^{b}A ± 4.90	43.0 ^{a}A ± 6.10
Yield	0.22 ^{d}A ± 0.31	0.32 ^{a}A ± 0.45	0.24 ^{c}A ± 0.36	0.29 ^{b}A ± 0.38
Dry See	0.80 ^{a}A ± 0.045	0.80 ^{a}A ± 0.038	0.80 ^{a}A ± 0.041	0.80 ^{a}A ± 0.042
Proteins	10.50 ^{a}B ± 1.80	11.30 ^{a}A ± 2.20	10.80 ^{a}A ± 2.10	10.60 ^{a}A ± 1.95
Dry Gluten	7.00 ^{a}B ± 1.20	7.40 ^{a}B ± 1.45	6.80 ^{a}B ± 1.25	6.90 ^{a}B ± 1.30
β-carotene	0.52 ^{a}B ± 0.085	0.63 ^{a}B ± 0.12	0.65 ^{a}B ± 0.11	0.64 ^{a}B ± 0.095
TP	20.50 ^{c}A ± 0.88	22.41 ^{ab}A ± 0.95	22.25 ^{b}A ± 0.93	22.54 ^{a}A ± 1.02
TF	9.48 ^{c}B ± 0.41	19.76 ^{a}B ± 0.82	17.55 ^{b}A ± 0.73	18.31 ^{a}B ± 0.77
DPPH	33.99 ^{b}A ± 1.25	34.95 ^{b}A ± 1.29	36.54 ^{a}A ± 1.31	37.09 ^{a}A ± 1.34
ABTS^{+}	24.13 ^{b}A ± 1.08	23.88 ^{b}A ± 1.15	25.08 ^{ab}A ± 1.17	26.29 ^{a}A ± 1.21
		2024		
Plant height	60.3 ^{c}B ± 7.80	89.6 ^{a}B ± 7.40	72.5 ^{b}B ± 5.30	87.7 ^{a}A ± 6.80
seed/ear	38.0 ^{b}A ± 5.20	53.0 ^{a}A ± 7.30	35.0 ^{b}A ± 4.60	51.0 ^{a}A ± 6.90
Yield	0.20 ^{b}A ± 0.19	0.33 ^{a}A ± 0.47	0.26 ^{a}A ± 0.27	0.30 ^{a}A ± 0.22
Dry Seed	0.80 ^{a}A ± 0.052	0.80 ^{a}A ± 0.046	0.80 ^{a}A ± 0.044	0.80 ^{a}A ± 0.049
Proteins	14.50 ^{a}A ± 2.60	14.80 ^{a}A ± 2.85	14.80 ^{a}A ± 2.75	15.20 ^{a}A ± 3.10
Dry Gluten	12.10 ^{a}A ± 2.20	12.00 ^{a}A ± 2.35	11.70 ^{a}A ± 2.00	11.80 ^{a}A ± 2.10
β-carotene	2.23 ^{a}A ± 0.28	2.31 ^{a}A ± 0.32	2.30 ^{a}A ± 0.29	2.34 ^{a}A ± 0.35
TP	11.47 ^{c}B ± 0.52	11.09 ^{c}B ± 0.58	21.19 ^{a}A ± 1.01	20.53 ^{a}B ± 0.96
TF	10.55 ^{c}A ± 0.46	20.80 ^{a}A ± 0.94	16.87 ^{b}A ± 0.88	23.17 ^{a}A ± 1.01
DPPH	14.95 ^{c}B ± 0.85	22.93 ^{b}B ± 1.17	35.21 ^{a}A ± 1.39	35.25 ^{a}A ± 1.26
ABTS^{+}	24.82 ^{b}A ± 1.10	25.80 ^{b}A ± 1.13	27.44 ^{a}A ± 1.24	28.55 ^{a}A ± 1.19

CTR (control) = soil without fertilizer; NPK = nitrogen–phosphorus–potassium; HM = horse manure; RecOrgFert = bentonite sulfur + orange pomace. Plant height (cm); Seed/ear; Yield (Kg/m^2); Dry Seed (g/plant); Proteins (%); Dry gluten (% s.s.); β-carotene (mg/100 g). TP = total phenols (mg GAE g^{-1}); TF = total flavonoids (mg QE g^{-1}); DPPH = 2.2-difenil-1-picrilidrazile (% inhibition); ABTS^{+} = 2.2′-azino-bis-3-etilbenzotiazolin-6-solfonato (% inhibition). Data are the means of three replicates ± standard deviation. Different lowercase letters in the same row indicate significant differences (Tukey's test. $p \leq 0.05$). Different uppercase letters in the same column indicate significant differences (Tukey's test. $p \leq 0.05$) for the same parameters between the different years.

In 2024 (Table 4), plant height and seed/ear values declined in the control plot but remained high in NPK and RecOrgFert plots. Yield was the highest in NPK (0.33 Kg/m^2), followed by RecOrgFert (0.30 Kg/m^2) and HM (0.26 Kg/m^2).

Protein and gluten values improved significantly in 2024. Protein content in wheat fertilized with RecOrgFert reached 15.20%, with dry gluten content of11.80%. These results indicate superior nitrogen assimilation and grain quality under RecOrgFert. The β-carotene content in grain with RecOrgFert increased drastically from ~0.61 mg/100 g in 2023 to over ~2.30 mg/100 g in 2024 across all treatments.

3.2.3. Grain Biochemical Quality

In 2023 (Table 4), RecOrgFert led in TP (22.54 mg GAE/g), DPPH (37.09%), and ABTS$^+$ (26.29%). In 2024 (Table 4), HM ranked highest for DPPH (35.21%) and RecOrgFert retained high TP and antioxidant values. TF increased notably in NPK and RecOrgFert (20.80 and 23.17 mg QE/100 g).

4. Discussion

The results obtained in Greece demonstrated that RecOrgFert—a sustainable fertilizer composed of sulfur-bentonite and orange processing residues—significantly improved both soil health and wheat grain quality under Mediterranean conditions. In contrast to synthetic NPK, which is linked to soil degradation, greenhouse gas emissions, and water pollution [2,3], RecOrgFert enhanced soil organic matter and moisture retention while consistently increasing wheat protein and gluten content. These effects are largely attributable to its sulfur component, a limiting nutrient in many Mediterranean soils that is essential for the biosynthesis of sulfur-containing amino acids (e.g., methionine and cysteine) and for efficient nitrogen assimilation, both of which support protein accumulation [28,29]. The addition of orange-derived organic matter further boosted RecOrgFert's effectiveness, as citrus residues are rich in polyphenols, flavonoids, and soluble carbon that stimulate microbial activity and serve as precursors for antioxidant biosynthesis [30]. Accordingly, RecOrgFert-treated grains showed consistently higher phenolic content, β-carotene levels, and antioxidant activity (DPPH, ABTS$^+$).

Pearson correlation analysis across the 2023–2024 trials provided further evidence of RecOrgFert's capacity to create a rhizosphere optimized to produce functional foods. The 2023 data revealed balanced interactions between nutrient availability and plant growth, leading to simultaneous improvements in yield and nutritional quality, while the 2024 findings confirmed these patterns, showing synchronized nutrient absorption and strong positive correlations among bioactive compounds. The progressive improvements observed between the two years suggest long-term benefits through structural changes in soil quality. Importantly, the consistency of results across different seasonal conditions underscores RecOrgFert as a reliable and sustainable fertilization strategy. These findings are in line with previous studies reporting that organic and organo-mineral fertilization enhances microbial diversity and nutrient cycling, with downstream effects on crop health and quality [31–33]. Overall, the combined contribution of sulfur and bioactive-rich citrus residues in RecOrgFert improved both soil biochemistry and grain nutritional quality, confirming its promise as a waste-derived fertilizer that can support agroecological transition strategies in Mediterranean drylands [6–8].

Comparable outcomes were observed in Southern Italy, where RecOrgFert not only enhanced wheat yield and grain quality but also improved key soil fertility parameters in a region prone to organic matter depletion and low microbial activity [5,34]. As in Greece, sulfur facilitated nitrogen assimilation and protein synthesis, leading to higher grain protein and gluten levels [28], while citrus residues stimulated microbial activity and contributed

to greater β-carotene and phenolic accumulation [8,35,36]. RecOrgFert also demonstrated consistency in yield and quality across years, suggesting resilience to seasonal variability. The Italian correlation analysis confirmed its superior effectiveness: in 2023, RecOrgFert fostered positive relationships between soil ΔpH and antioxidant activity, as well as optimization of Δβ-carotene profiles, indicating synergistic coordination between nutrient absorption and bioactive compound synthesis. By 2024, these effects had strengthened, with clearer patterns of carotenoid optimization and more robust inter-nutrient correlations, highlighting progressive improvements not seen with conventional treatments.

Taken together, the results from both sites confirm RecOrgFert's dual role in enhancing soil fertility and boosting crop nutritional value, with effects that consolidate over time. The agreement between Greek and Italian trials underscores its reliability across contrasting Mediterranean environments, supporting its application as a practical and resilient fertilization strategy. Consistent with recent literature, these findings emphasize the potential of organic and organo-mineral amendments to improve soil physicochemical properties, microbial diversity, and ultimately the functional quality of food crops [31–38]. By integrating sulfur and bioactive-rich residues into a single formulation, RecOrgFert emerges as a particularly effective approach to producing high-value, nutrient-dense grains while contributing to the sustainability of Mediterranean agroecosystems.

5. Conclusions

This study provides compelling evidence that RecOrgFert is a highly effective and sustainable solution for Mediterranean wheat systems. Across two growing seasons and two agro-ecological contexts (Greece—Central Macedonia and Italy—Apulia), RecOrgFert demonstrated the ability to enhance soil fertility, increase plant productivity, and improve grain nutritional and functional quality. Unlike synthetic fertilizers such as NPK, RecOrgFert promoted organic matter accumulation and improved soil chemical balance (pH, EC, WC). These improvements translated into agronomic outcomes, including higher yields, improved nitrogen use efficiency, and significant enhancement of protein, gluten, antioxidant, and β-carotene content in wheat grain. RecOrgFert is not merely a viable alternative to mineral fertilizers—it is a strategic resource for advancing sustainable, circular, and climate-resilient agriculture in the Mediterranean and beyond. Future research should build on the present findings to consolidate RecOrgFert's role as both a scientific innovation and a practical solution at the nexus of productivity, sustainability, and food security. Future research prospects and considerations should focus on long-term trials across different soil types, climates, and crop systems to assess its capacity to restore degraded soils. Mechanistic studies on soil–microbiome interactions are also needed to provide deeper insights into how citrus-derived organic fractions stimulate beneficial microbial consortia and influence nutrient cycling.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app151810292/s1, Table S1: Seasonal Climate in Thessaloniki (2023/2024 Overview, based on averages and reported events). Table S2: Seasonal Climate in Foggia (Apulia, Italy—2023/2024 Overview based on averages and reported events). Figure S1: (a) Experimental field Apulia south Italy, control (Red), NPK (Green), RecOrgFert (Yellow), and horse manure (Blue). (b) experimental field Thessaloniki Greece, Control (green), NPK (blue), RecOrgFert (Yellow), and horse manure (violet). Figure S2: Pearson correlation matrix illustrating the relationships between soil and grain parameters for the experiment in Greece 2023. Figure S3: Pearson correlation matrix illustrating the relationships between soil and grain parameters in Greece 2024. Figure S4: Pearson correlation matrix illustrating the relationships between soil and grain parameters in Italy 2023. Figure S5: Pearson correlation matrix illustrating the relationships between

soil and grain parameters in Italy 2024. Table S3: Chemical Characteristics of Soils in Greece and Apulia before starting the experiments (T0).

Author Contributions: Conceptualization, A.M. (Adele Muscolo), A.M. (Angela Maffia) and E.V.; methodology, S.B.; software, L.S. and M.O.; validation, A.M. (Adele Muscolo) and A.M. (Angela Maffia); formal analysis, C.M., S.B., F.A.M. and K.Z.; investigation, F.A.M., S.B., K.Z. and C.M.; resources, A.M. (Adele Muscolo); data curation, K.Z., L.S. and M.O.; writing—original draft preparation, A.M. (Adele Muscolo) and M.O.; writing—review and editing, A.M. (Adele Muscolo) and A.M. (Angela Maffia); visualization, M.O. and L.S.; supervision, A.M. (Adele Muscolo); project administration, A.M. (Adele Muscolo); funding acquisition, A.M. (Adele Muscolo). All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by European Commission, project LIFE20ENV/IT/000,229–LIFE RecOrgFert PLUS, LIFE Programme.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CTR	Control, soil without fertilizer
NPK	Nitrogen–phosphorus–potassium
HM	Horse manure
WC	Water content
EC	Electrical conductivity
OC	Organic carbon
TotN	Total nitrogen
C/N	Carbon–nitrogen ratio
OM	Organic matter
CEC	Cation exchange capacity
TP	Total phenols
TF	Total flavonoids
DPPH	2,2-difenil-1-picrilidrazile
$ABTS^{+}$	2,2′-azino-bis-3-etilbenzotiazolin-6-solfonato

References

1. FAO FAOSTAT Statistical Database. Available online: https://www.fao.org/faostat/en/#home (accessed on 22 July 2025).
2. Zhang, Y.-J.; Gan, R.-Y.; Li, S.; Zhou, Y.; Li, A.-N.; Xu, D.-P.; Li, H.-B. Antioxidant Phytochemicals for the Prevention and Treatment of Chronic Diseases. *Molecules* **2015**, *20*, 21138–21156. [CrossRef] [PubMed]
3. Zhang, L.; Zhao, Z.; Jiang, B.; Baoyin, B.; Cui, Z.; Wang, H.; Li, Q.; Cui, J. Effects of Long-Term Application of Nitrogen Fertilizer on Soil Acidification and Biological Properties in China: A Meta-Analysis. *Microorganisms* **2024**, *12*, 1683. [CrossRef] [PubMed]
4. Ali, A.; Jabeen, N.; Farruhbek, R.; Chachar, Z.; Laghari, A.A.; Chachar, S.; Ahmed, N.; Ahmed, S.; Yang, Z. Enhancing Nitrogen Use Efficiency in Agriculture by Integrating Agronomic Practices and Genetic Advances. *Front. Plant Sci.* **2025**, *16*, 1543714. [CrossRef] [PubMed]
5. Diacono, M.; Montemurro, F. Long-Term Effects of Organic Amendments on Soil Fertility. In *Sustainable Agriculture Volume 2*; Lichtfouse, E., Hamelin, M., Navarrete, M., Debaeke, P., Eds.; Springer: Dordrecht, The Netherlands, 2011; pp. 761–786. ISBN 978-94-007-0394-0.
6. Badagliacca, G.; Testa, G.; La Malfa, S.G.; Cafaro, V.; Lo Presti, E.; Monti, M. Organic Fertilizers and Bio-Waste for Sustainable Soil Management to Support Crops and Control Greenhouse Gas Emissions in Mediterranean Agroecosystems: A Review. *Horticulturae* **2024**, *10*, 427. [CrossRef]
7. Mulatu, G.; Bayata, A. Vermicompost as Organic Amendment: Effects on Some Soil Physical, Biological Properties and Crops Performance on Acidic Soil: A Review. *FEM* **2024**, *10*, 66–73. [CrossRef]

8. Lazcano, C.; Boyd, E.; Holmes, G.; Hewavitharana, S.; Pasulka, A.; Ivors, K. The Rhizosphere Microbiome Plays a Role in the Resistance to Soil-Borne Pathogens and Nutrient Uptake of Strawberry Cultivars under Field Conditions. *Sci. Rep.* **2021**, *11*, 3188. [CrossRef]
9. European Commission. *Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions: A New Circular Economy Action Plan—For a Cleaner and More Competitive Europe*; European Commission: Brussels, Belgium, 2020.
10. Montemurro, F.; Maiorana, M.; Convertini, G.; Ferri, D. Compost Organic Amendments in Fodder Crops: Effects on Yield, Nitrogen Utilization and Soil Characteristics. *Compost Sci. Util.* **2006**, *14*, 114–123. [CrossRef]
11. Pelman, A.; Vries, J.W.D.; Tepper, S.; Eshel, G.; Carmel, Y.; Shepon, A. A Life-Cycle Approach Highlights the Nutritional and Environmental Superiority of Agroecology over Conventional Farming: A Case Study of a Mediterranean Farm. *PLoS Sustain. Transform.* **2024**, *3*, e0000066. [CrossRef]
12. Fiorentini, M.; Schillaci, C.; Denora, M.; Zenobi, S.; Deligios, P.A.; Santilocchi, R.; Perniola, M.; Ledda, L.; Orsini, R. Fertilization and Soil Management Machine Learning Based Sustainable Agronomic Prescriptions for Durum Wheat in Italy. *Precis. Agric.* **2024**, *25*, 2853–2880. [CrossRef]
13. Walkley, A.; Black, I.A. An Examination of the Degtjareff Method for Determining Soil Organic Matter, and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
14. Kjeldahl, J. New Method for the Determination of Nitrogen. *Sci. Am.* **1883**, *48*, 101–102. [CrossRef]
15. Kaminsky, R.; Muller, W.H. The Extraction of Soil Phytotoxins Using a Neutral EDTA Solution. *Soil Sci.* **1977**, *124*, 205. [CrossRef]
16. Kaminsky, R.; Müller, W.H. A Recommendation against the Use of Alkaline Soil Extractions in the Study of Allelopathy. *Plant Soil* **1978**, *49*, 641–645. [CrossRef]
17. *ISO 10390*; Soil, Treated Biowaste and Sludge. Determination of pH ISO (International Organization for Standardization): Geneva, Switzerland, 2021.
18. *ISO 14240*; Soil Quality—Determination of Soil Microbial Biomass—Part 1: Substrate-Induced Respiration Method. ISO: Geneva, Switzerland, 1997.
19. AACC. *Approved Methods of the American Association of Cereal Chemists*; American Association of Cereal Chemists: St. Paul, MN, USA, 2000.
20. Dowell, F.E.; Maghirang, E.B.; Xie, F.; Lookhart, G.L.; Pierce, R.O.; Seabourn, B.W.; Bean, S.R.; Wilson, J.D.; Chung, O.K. Predicting Wheat Quality Characteristics and Functionality Using Near-Infrared Spectroscopy. *Cereal Chem.* **2006**, *83*, 529–536. [CrossRef]
21. Weegels, P.L.; Hamer, R.J.; Schofield, J.D. Functional Properties of Wheat Glutenin. *J. Cereal Sci.* **1996**, *23*, 1–17. [CrossRef]
22. Saini, R.K.; Prasad, P.; Lokesh, V.; Shang, X.; Shin, J.; Keum, Y.-S.; Lee, J.-H. Carotenoids: Dietary Sources, Extraction, Encapsulation, Bioavailability, and Health Benefits—A Review of Recent Advancements. *Antioxidants* **2022**, *11*, 795. [CrossRef] [PubMed]
23. Box, J.D. Investigation of the Folin-Ciocalteau Phenol Reagent for the Determination of Polyphenolic Substances in Natural Waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
24. Velioglu, Y.S.; Mazza, G.; Gao, L.; Oomah, B.D. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products. *J. Agric. Food Chem.* **1998**, *46*, 4113–4117. [CrossRef]
25. Djeridane, A.; Yousfi, M.; Nadjemi, B.; Boutassouna, D.; Stocker, P.; Vidal, N. Antioxidant Activity of Some Algerian Medicinal Plants Extracts Containing Phenolic Compounds. *Food Chem.* **2006**, *97*, 654–660. [CrossRef]
26. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef] [PubMed]
27. Barreca, D.; Bellocco, E.; Caristi, C.; Leuzzi, U.; Gattuso, G. Flavonoid Composition and Antioxidant Activity of Juices from Chinotto (Citrus × Myrtifolia Raf.) Fruits at Different Ripening Stages. *J. Agric. Food Chem.* **2010**, *58*, 3031–3036. [CrossRef] [PubMed]
28. Castellari, M.P.; Poffenbarger, H.J.; Van Sanford, D.A. Sulfur Fertilization Effects on Protein Concentration and Yield of Wheat: A Meta-Analysis. *Field Crops Res.* **2023**, *302*, 109061. [CrossRef]
29. Narayan, O.P.; Kumar, P.; Yadav, B.; Dua, M.; Johri, A.K. Sulfur Nutrition and Its Role in Plant Growth and Development. *Plant Signal. Behav.* **2023**, *18*, 2030082. [CrossRef]
30. Bustamante, M.A.; Moral, R.; Paredes, C.; Pérez-Espinosa, A.; Moreno-Caselles, J.; Pérez-Murcia, M.D. Agrochemical Characterisation of the Solid By-Products and Residues from the Winery and Distillery Industry. *Waste Manag.* **2008**, *28*, 372–380. [CrossRef]
31. Shu, X.; He, J.; Zhou, Z.; Xia, L.; Hu, Y.; Zhang, Y.; Zhang, Y.; Luo, Y.; Chu, H.; Liu, W.; et al. Organic Amendments Enhance Soil Microbial Diversity, Microbial Functionality and Crop Yields: A Meta-Analysis. *Sci. Total Environ.* **2022**, *829*, 154627. [CrossRef]
32. Dincă, L.C.; Grenni, P.; Onet, C.; Onet, A. Fertilization and Soil Microbial Community: A Review. *Appl. Sci.* **2022**, *12*, 1198. [CrossRef]

33. Zhang, Z.; He, P.; Hao, X.; Li, L.-J. Long-Term Mineral Combined with Organic Fertilizer Supports Crop Production by Increasing Microbial Community Complexity. *Appl. Soil Ecol.* **2023**, *188*, 104930. [CrossRef]
34. Pei, B.; Liu, T.; Xue, Z.; Cao, J.; Zhang, Y.; Yu, M.; Liu, E.; Xing, J.; Wang, F.; Ren, X.; et al. Effects of Biofertilizer on Yield and Quality of Crops and Properties of Soil Under Field Conditions in China: A Meta-Analysis. *Agriculture* **2025**, *15*, 1066. [CrossRef]
35. Wang, Y.; Li, Q.; Li, C. Organic Fertilizer Has a Greater Effect on Soil Microbial Community Structure and Carbon and Nitrogen Mineralization than Planting Pattern in Rainfed Farmland of the Loess Plateau. *Front. Environ. Sci.* **2023**, *11*, 1232527. [CrossRef]
36. Basharat, Z.; Basharat, B.; Noor, M.; Batool, Z. Citrus Waste in Agriculture: Fertilizers and Soil Amendments. In *Valorization of Citrus Food Waste*; Chauhan, A., Islam, F., Imran, A., Singh Aswal, J., Eds.; Springer Nature: Cham, Switzerland, 2025; pp. 335–349. ISBN 978-3-031-77999-2.
37. Ma, Y.; Zhang, S.; Feng, D.; Duan, N.; Rong, L.; Wu, Z.; Shen, Y. Effect of Different Doses of Nitrogen Fertilization on Bioactive Compounds and Antioxidant Activity of Brown Rice. *Front. Nutr.* **2023**, *10*, 1071874. [CrossRef] [PubMed]
38. Nowak, R.; Szczepanek, M.; Kobus-Cisowska, J.; Stuper-Szablewska, K.; Dziedziński, M.; Błaszczyk, K. Profile of Phenolic Compounds and Antioxidant Activity of Organically and Conventionally Grown Black-Grain Barley Genotypes Treated with Biostimulant. *PLoS ONE* **2023**, *18*, e0288428. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli

Angela Maffia[1], Federica Marra [1], Santo Battaglia[1], Mariateresa Oliva[1] , Carmelo Mallamaci[1] and Adele Muscolo [1]*

[1]Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy;

* Correspondence: amuscolo@unirc.it; Tel.: +39-09651694364

Soil Systems

https://doi.org/10.3390/soilsystems8020053

Received: 9 April 2024

Revised: 6 May 2024

Accepted: 9 May 2024

Published: 15 May 2024

soil systems

MDPI

Article

Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli

Angela Maffia, Federica Marra, Santo Battaglia, Mariateresa Oliva, Carmelo Mallamaci and Adele Muscolo *

Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); federica.marra@unirc.it (F.M.); battaglia.santo96@gmail.com (S.B.); mariateresa.oliva@unirc.it (M.O.); carmelo.mallamaci@unirc.it (C.M.)
* Correspondence: amuscolo@unirc.it; Tel.: +39-09651694364

Abstract: In this work, environmentally sound technologies for converting organic wastes into fertilizers to improve soil sustainability and crop yield have been identified and assessed. Wet wastes were combined with 50% wood sawdust and 50% wet wastes (Compost 1) or (10% Straw + 90% wet wastes) (Compost 2) to produce soil improvers with a balanced level of nutrients, and their effectiveness on soil ecosystem functioning have been tested and compared to horse manure (HM) and nitrogen–phosphorous–potassium (NPK) fertilizers. Unfertilized soil was used as a control. Soil chemical and biological properties have been detected after the harvesting of broccoli and red cabbage (90 days from the initial treatments). Three independent experiments have been conducted in an open field in a randomized complete block design with three replications (n = 9). The results showed that Compost 1 had the highest C/N ratio and cation exchange capacity (CEC), indicating a better humification of the wet material. Compost 1, even if it contained a minor amount of organic carbon, as well as less activity of fluorescein diacetate (FDA) and dehydrogenase (DHA) than Compost 2, was the most effective in improving soil quality, significantly increasing the labile fraction of organic matter, the oxidative enzyme (DHA), microbial biomass, and crop yield. Both composts increased crop productivity.

Keywords: waste compost; soil fertility; broccoli calabrese; red cabbage; soil amendments

check for updates

Citation: Maffia, A.; Marra, F.; Battaglia, S.; Oliva, M.; Mallamaci, C.; Muscolo, A. Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli. *Soil Syst.* **2024**, *8*, 53. https://doi.org/10.3390/soilsystems8020053

Academic Editors: Douglas Guelfi, Heitor Cantarella and Flávio Henrique Silveira Rabêlo

Received: 9 April 2024
Revised: 6 May 2024
Accepted: 9 May 2024
Published: 15 May 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

In the face of global challenges, such as population growth, climate change, and diminishing agricultural resources, there is an increasing imperative to develop sustainable agricultural practices that simultaneously enhance soil fertility, mitigate waste-disposal issues, and reduce environmental impacts [1]. Based on the latest UN projections, the world's population may rise to roughly 8.5 billion by 2030, 9.7 billion by 2050, and could peak around 10.4 billion in the 2080s. Consequently, our yearly food supply needs to sustainably meet the demands of this growing populace [2]. Sustainable agricultural methods offer ways to produce food and other agricultural products with minimal environmental impact. This ensures consistent food access and availability and environmental and human health safeguards. Sustainable agriculture is linked to food security, which encompasses consistent food availability; adequate production; affordability; sufficient nutrition in terms of energy, proteins, and micronutrients; safety; and the economic stability to maintain these factors [3]. It is imperative to identify and analyze well-established approaches aimed at fostering sustainable agriculture, many of which prioritize ecosystem health.

These approaches are characterized by clear principles and encompass environmental, economic, and social objectives. They have evolved as methodological strategies over time, like agroecology and sustainable intensification, or were prioritized from the outset, such as carbon farming.

Composting represents a promising solution, offering a means to recycle organic materials while generating nutrient-rich soil amendments [4]. In essence, composts derived from waste management within the context of the circular economy, such as biowaste, organic waste, and green waste, are considered acceptable for use in organic agriculture under Regulation EU 2021/1165 [5].

This allowance is contingent upon these composts originating from a recognized separate collection system within the respective EU member state and adhering to specified limits for heavy metal content. Compost, when used as a fertilizer or soil conditioner, can significantly enhance soil quality [6,7]. It accomplishes this by improving aeration, optimizing water content, enhancing aggregate stability, and thereby bolstering resistance against erosion. Furthermore, compost enriches the soil with both macro and micronutrients, fostering healthier plant growth, and augments the cation exchange capacity, as demonstrated by Muscolo et al. (2018) [6] and Ghimire et al. (2023) [8]. Activating the soil microbiota and increasing its biomass are additional benefits, although the extent of these effects relies heavily on the quality and quantity of organic matter, as observed by Bonanomi et al. (2020) [9] and Sunman et al. (2022) [10]. When it comes to the risk of nitrate leaching, composted organic waste is generally considered to pose minimal risk [11].

This manuscript explores the scientific dimensions of composting through the lens of a specific approach: the utilization of wood sawdust and vegetable wastes as composting materials.

The selection of wood sawdust and vegetable wastes for composting is rooted in their unique compositional characteristics. Wood sawdust, a by-product of various woodworking processes, is recognized for its high carbon content and lignocellulosic structure [12]. This provides an excellent source of carbon, crucial for establishing an optimal carbon-to-nitrogen ratio in the composting process. Additionally, wood sawdust represents to the wood industry a waste to be disposed of with economic implications. In contrast, vegetable wastes, including kitchen scraps and garden trimmings, contribute nitrogen-rich organic matter. When these materials are co-composted, they hold the potential to create a well-balanced mixture, essential for the efficient decomposition of organic matter [13].

The science of composting hinges upon the microbial-driven biological transformation of organic materials into stabilized organic matter known as humus. This process involves a complex interplay of organisms, including bacteria, fungi, actinomycetes, and earthworms that break down the organic compounds present in the feedstock. In the case of wood sawdust and vegetable-waste composting, the intricate lignocellulosic structure of sawdust provides an intriguing substrate for microbial colonization and degradation, leading to the release of carbon and other nutrients [14].

The resulting compost, characterized by a dark, crumbly texture, not only sequesters carbon but also embodies essential nutrients such as nitrogen, phosphorus, and potassium, as well as micronutrients required for plant growth. Beyond its nutrient content, the compost enhances soil structure, moisture retention, and microbial diversity, ultimately fostering improved soil health and agricultural productivity. Furthermore, this manuscript a part to delve into the scientific aspects of composting management, addressing critical factors such as temperature dynamics, aeration, moisture content, and composting timeframes, explore the effects of compost as fertilizer on broccoli and cabbage growth and yield. Particularly, the growth parameters related to productivity and the parameters related to plant performance have been detected and discussed. This manuscript explores the scientific intricacies of this composting method, shedding light on its potential to transform wastes into a valuable resource, mitigate greenhouse-gas emissions, and enhance soil fertility and crop yield, and provides a compelling avenue toward a more sustainable and resilient agricultural future.

2. Materials and Methods

2.1. Feeding Materials

The raw organic materials employed for composting comprised a variety of components and precise vegetable wastes (like rocket salad, lettuce, cabbage, carrots, and valerian).

The two composts have been prepared using different percentages of the vegetable residues (Table 1).

Table 1. Compostable raw materials of different composts used.

Compost ID	Compostable Raw Material
Compost 1 (C1)	50% wood sawdust + 50% wet waste, such as kitchen and restaurant scraps.
Compost 2 (C2)	10% Straw + 90% wet wastes, such as kitchen and restaurant scraps.

2.2. Composting Process Setup

Sawdust and vegetable residues, as well as straw and vegetable residues, were carefully deposited into dedicated electric composters and subjected to the composting process. The electric composter contains multiple chambers for a better composting process. The waste in the second chamber does not come into contact with the fresh waste; the temperatures of the two chambers are managed independently for the better development of microbial populations. The tank dimensions were 350 cm × 100 cm × 131 cm and had a capacity of 10 tons/year. This composting protocol was replicated three times for each compost mixture. The composting conditions were controlled as follows: an initial mesophilic phase for 8 days at 29 °C followed by a thermophilic phase lasting 20 days at 50 °C.

A second mesophilic phase extended for 92 days at 27 °C. The temperature increases during the composting process resulted from the robust microbial activity, facilitated by efficient ventilation within the mixture.

The ventilation process is maintained and accelerated by the continuous supply of air and the continuous movement of the organic material. This ventilation process guaranteed the presence of ample oxygen levels, thereby promoting biological processes while maintaining optimal aerobic conditions, as documented by Liang et al. (2003) [15]. Following this mesophilic phase, a stabilization transitioning phase with a stable temperature of 20 °C for 30 days was settled to stabilize the compost until the conclusion of the composting cycle. This stability was attributed to reduced microbial activity and a diminishing quantity of organic substrate available for decomposition. The moisture content was upheld at 50%, and the oxygen percentage consistently exceeded 15%. Temperature, moisture, and oxygen levels were monitored daily using a probe strategically placed in the center of the composting mass, ensuring they remained within the predefined parameters. Water was added as needed to sustain the 50% moisture level. Daily agitation of the mixtures was performed to guarantee oxygen levels above 15%, thereby promoting the aerobic decomposition of organic matter into stable humus. Comprehensive decomposition and stabilization of the materials were accomplished over a span of 4 months. Subsequently, all compost batches underwent an air-drying process, were finely crushed to pass through a 2 mm sieve, and underwent thorough blending to ensure uniformity.

2.3. Chemical Characterization of Composts

The chemical analysis of the composts was conducted in accordance with the protocols outlined in the ANPA manual from 2001 [16]. To evaluate the rate of organic matter mineralization, the reduction in organic matter content over time was assessed by using the following equation (Equation (1)):

$$\text{Organic matter loss (\%)} = [(\text{Initial mass of carbon} - \text{Final mass of carbon})/\text{Initial mass of carbon}] \times 100 \quad (1)$$

The determination of fluorescein 3,6-diacetate hydrolase activity followed the procedure established by Adam and Duncan [17]. The results were expressed as mg fluorescein released per gram of dry soil, following Perucci et al. (1992) [18].

Dehydrogenase (DHA) activity was determined as outlined by von Mersi and Schinner (1991) [19]. The absorbance of the soil filtrate was measured at 490 nm.

Water-soluble phenols (WSP) were detected by extracting the soil with water and determining their concentration using the Folin–Ciocalteau reagent, following Box's method from 1983 [20]. The cation exchange capacity (CEC) was determined using an aqueous solution of $BaCl_2$ buffered to pH 7.0 to saturate the soil-exchange complex, following Hendershot and Duquette (1986) [21].

Compost maturity was assessed by employing cucumber (*Cucumis sativus* L.) seeds, following the method described by Gariglio et al. (2002) [22]. The Germination Index (GI), which combines measures of relative seed germination (%) and relative root elongation (%), was used to evaluate compost toxicity. This method is particularly sensitive and capable of detecting both low-level and high-level toxicity affecting root growth and germination, respectively. A GI value higher than 60% indicates the non-phytotoxicity of the compost, as established by Zucconi et al. (1981) [23].

The organic matter mineralization rate was assessed by evaluating the loss of organic matter over time, Organic matter loss was calculated following Equation (1).

The absorption capacity of the composts, in relation to Na^+ and Cl^- ions, has been calculated using the following Formula (2):

$$AC = (Ci - Cf)\ m \times VAC = m(Ci - Cf) \times V \quad (2)$$

where:

- AC is absorption capacity;
- Ci is the initial concentration of ions;
- Cf is the final concentration of ions;
- m is the compost weight;
- V is the volume of the solution (40 mL).

2.4. Soil Characteristics and Treatments

The experiment was carried out in sandy-loam soil belonging to Cambisol (WRB, 2022) [24], located in Motta San Giovanni, Loc. Liso, Italy (37.9991° N. 15.6999° E).

The average temperature of the coldest months, January and February, stands at +11.9 °C; that of the hottest month, August, is +26.1 °C. The average annual precipitation is around 493 mm, with a minimum in summer and a peak in autumn–winter. The fertilization experiments consisted of three replicate plots for each condition. Each plot measured 18 m^2 and was set up using a single-factor randomized complete block design. The soil received a fertilization treatment using the two composts distributed at a depth of 10/15 cm. In each designated plot, composts were incorporated based on the organic matter content precisely at rates of 3.1 Mg/ha for composts, horse manure (HM, 4.3 Mg/ha), and NPK (20:10:10) at 1.7 Mg/ha. To maintain consistent moisture levels, the plants were regularly irrigated to ensure a water content of 70% of field capacity across all parcels. The experiment was replicated three times. Two different crops have been used to test the effectiveness of the two produced composts, namely ramous Calabria broccoli and red cabbage.

The differently treated crops were collected when they reached ripeness level, based on visual characteristics such as size, shape, and color. Cabbage cultivated with Compost 1 matured in a range of 78 days, while those grown with Compost 2 maturated in 85 days, with HM in 88 days, and with NPK taking 90 days to mature. Broccoli was ready to be harvested 70 days after transplanting when cultivated with Compost 1, 79 days when cultivated with Compost 2, 83 days when grown with NPK, and 80 days with HM. Within each plot, for both crops (broccoli and cabbage), 3–4 plants/m^2 were planted for each treatment. The spacing between individual plants was set at 40 cm, with 60 cm between rows. Throughout the experiment, the parcels were irrigated to maintain the soil moisture at 70% of field capacity. Soil humidity was continuously monitored using a direct-read soil pH/moisture meter—R181 to ensure consistent soil moisture levels in both soil types.

2.5. Soil Analysis

Soils were collected in each parcel at the end of the experiment (90 days), as reported below for the specific crop species and fertilization used. Soils have been air-dried and sieved through a 2 mm sieve for chemical analyses, while fresh soil sieved to 2 mm was used for microbiological analyses. The soil water content was expressed in percentage. The water content was determined at the beginning of the experiment and every 15 days during the entire experiment for all soil treatments. Particle-size analysis was carried out by using the method of Bouyoucos et al. (1962) [25]. Dry matter (dm) was determined by weighing the samples after 24 h at 105 °C; pH and EC were measured as reported in Muscolo et al. (2017) [26]. Organic carbon was determined by oxidimetric method following the Walkley–Black procedure [27]; total nitrogen was determined by the digestion procedure, using sulfuric acid at temperatures of 380 °C following the method of Kjeldahl et al. (1883) [28]. The amount of microbial biomass carbon (MBC) was determined by using the chloroform fumigation extraction procedure [29] with field-moist samples (equivalent to 20 g dry wt.). The filtered soil extracts of both fumigated and unfumigated samples were analyzed for soluble organic C using the methods of Walkley and Black [27]. Microbial biomass C was estimated on the basis of the differences between the organic C extracted from the fumigated soil and that from the unfumigated soil, and an extraction efficiency coefficient of 0.38 was used to convert soluble C into biomass C [29]. The microbial population was extracted following the method of Insam and Goberna [30]. Two grams of soil and 30 glass beads were mixed with 20 mL 0.90% NaCl and shaken for 20 min at 20 °C, then centrifuged at 4 °C for 1 h at 12,000× *g* to separate the bacteria from the solid particles. The supernatant was used for further dilutions with a sterile one-fourth-strength Ringer solution so as to standardize the inoculum density. The soil's bacterial population was estimated by Waksman et al. (1952) [31] with method using the nutrient agar medium at 10^5 dilutions. The fungal population was estimated by the dilution-plate method using Martin's Rose Bengal agar medium at 10^3 dilutions in water [32]. The activities of fluorescein 3,6-diacetate hydrolase (FDA), and dehydrogenase (DHA), as well as the water-soluble phenol amount, ion concentrations, and cationic exchange capacity (CEC), were determined as reported in Section 2.3. Three soil samples for each crop and each specific fertilization have been collected. All the analyses were performed in triplicates. Thus, for each cultivar and condition, n = 9.

2.6. Crop Growth Assessment

Each cultivar was analyzed for the following growth parameters: plant height (PH) from the soil level to the highest point of the plant, leaf area (LA, cm^2), leaf length (LL, cm), leaf width (LW, cm), leaf humidity (LH, %) from the basal leaves to the last open leaf, fruit-head diameter (HD, cm) and yield (tons/hectare). For the estimation of total chlorophyll content, 100 mg of leaf tissue were finely ground in liquid nitrogen and suspended in dimethyl sulfoxide (DMSO). The suspension was maintained at 65 °C for 30 min. The final volume was adjusted to 10 mL with DMSO, and the absorbance was recorded at 645 and 663 nm. The total chlorophyll content was calculated as reported by Hiscox et al. (1979) [33].

2.7. Mineral Assay

Cations (Na, K, Ca, Mg) were extracted from samples and analyzed using ion chromatography (DIONEX ICS-1100, Thermo Fisher Scientific, Waltham, MA, USA). One g of dry material was ashes at 550 °C for 6 h in a porcelain capsule. The ash was then acidified for 30 min at 100 °C using 1M of HCl solution (10 mL). Finally, it was filtered using Whatman 1 and measured using the ion chromatograph with 20 mM methane–sulfonic acid as an eluent. The Fe concentration was determined using atomic absorption spectrophotometry (model 2380, PerkinElmer Co., Waltham, MA, USA). Four mixed standard solutions with concentrations of 1, 5, 25, and 50 ppm of each of the four desired anions were used to plot the calibration curve. The linear relationship between the peak area and concentration

was confirmed experimentally. The amount of each cation was calculated using its own standard curve. The amount of each anion was calculated using ion chromatography (DIONEX) and comparing the results with a multi-ion cation standard curve (Multi Ion Cation IC standard solution, Specpure®, Dionex, Thermo Fisher, Milan, Italy) [34].

All solvents and reagents were purchased from Panreac (Barcelona, Spain). The bioconcentration factor (cation or anion in root/cation or anion in soil), bioaccumulation coefficient (cation or anion in leaves/cation or anion in soil), and translocation factor (cation or anion in leaves/cation or anion in roots) were detected.

2.8. Chlorophyll Fluorescence Imaging

The photosynthetic efficiency of cabbage and broccoli leaves, differently fertilized, was evaluated by using an Imaging PAM Fluorometer (Walz). The chlorophyll fluorescence parameters detected were as follows: maximum quantum yield of PSII photochemistry (Fv/Fm); effective quantum yield of PSII photochemistry (Y(II)); quantum yield of regulated energy dissipation at PSII (Y(NPQ)); quantum yield of non-regulated energy dissipation at PSII (Y(NO)); non-photochemical quenching coefficient (NPQ), and electron transport rate (ETR). The maximum PSII quantum yield (Fv/Fm), photochemical fluorescence quenching (qP), non-photochemical fluorescence quenching (NPQ), and ETR have been evaluated and analyzed for the indication they give. Fv/Fm showed the maximum efficiency of energy conversion in PSII. qP indicates the rate of photochemical reactions in the chloroplast electron transport chain in vivo. NPQ indicates the amount of excess energy that was absorbed by chlorophyll but was not used by the electron transport chain and was converted to heat [35]. The ETR electron transport rate is proportional to the photosynthetic activity, and a higher value indicates higher carbon fixation activity. These parameters are measured in relative units.

2.9. Statistical Analyses

Data are expressed as the means of three analyses for each treatment and three analyses for different compost analyses. Significant difference tests were carried out to analyze the effects of fertilizers on each of the various parameters measured. Homogeneity of variance and normality were tested, respectively, with the tests by Bartlett and Shapiro with a p value of 0.05. For all other variables, a one-way ANOVA ($p < 0.05$) was performed, followed by Tukey's post hoc test tests, to find significant differences between treatments ($p < 0.05$). The ANOVA and t-test were carried out using XLStat. To explore the relationships among different fertilizers on the soil, broccoli, and red cabbage, we analyzed parameter datasets using principal component analysis (PCA) with XLStat.

3. Results

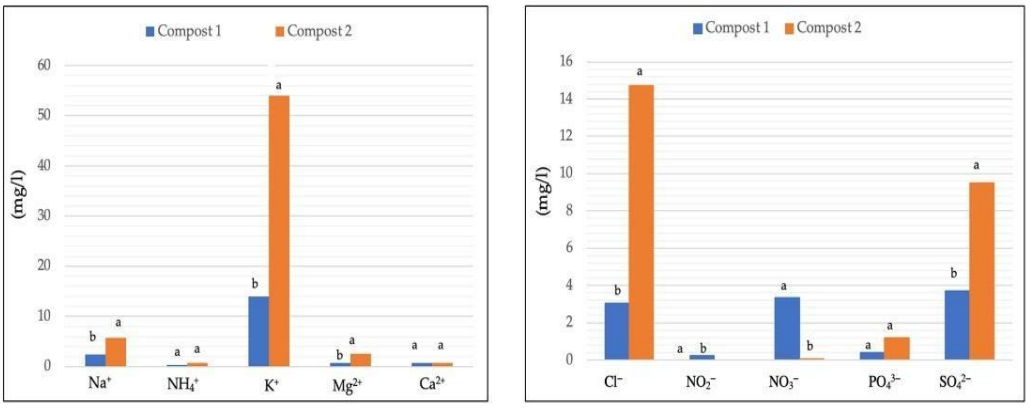
3.1. Compost Properties

The composting procedure underwent three repetitions through independent experiments. The outcomes consistently revealed that each compost derived from these experiments exhibited identical chemical characteristics. This observation strongly indicated that the adopted procedure has been successfully standardized, ensuring the reproducibility of the results over time. After a 4-month composting period, the analysis revealed noteworthy distinctions between the two composts obtained using the same methodology (Table 2). Both C1 and C2 composts displayed highly alkaline pH levels. C2 exhibited the highest total organic carbon and total nitrogen, while C1 and C2 differed in their C/N ratio (21.57 for C1 and 11.97 for C2). The $N\text{-}NH_4^+/N\text{-}NO_3^-$ ratio was the highest in C2, whereas the ON/TN ratio was significantly greater in C1 than in C2 (Table 2). Despite all composts being nutrient-rich (Figure 1a,b), C2 contained more nutrients than C1, in particular potassium and magnesium (Figure 1a), C1 contained the highest amount of NO_2 and NO_3. Conversely, C2 had the greatest amount of phosphates and sulfates (Figure 1b). Notably, C2 contained eight times more water-soluble phenols (WSP) and concurrently exhibited a greater cation exchange capacity and FDA and DHA activities than C1 (Figure 2). Assessing compost

maturity through phytotoxicity, as indicated by the germination index (Figure 3), revealed that C1 did not exhibit phytotoxicity in *Cucumis sativus*. The germination index measured 6 days post-germination at 25%, 50%, and 75% compost concentrations consistently exceeded 80%, classifying it as phytonutrient. These findings align with the overall germination index, ranging from 67.5% to 95%, confirming the non-phytotoxic nature of both composts.

Table 2. Physico-chemical properties of the two composts obtained from different raw materials Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process. pH (H_2O and KCl); electric conductivity (EC, mS cm^{-1}); water content (WC, %); total organic carbon (TOC, %); Total Nitrogen (TN, %); carbon–nitrogen ratio (C/N); ammonium-nitrogen–nitrate-nitrogen ratio (NH_4^+-N/NO_3^--N); organic nitrogen–total nitrogen ratio (ON/TN, %), water-soluble phenols (WSP µg GAE g^{-1} d.s). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences at $p \leq 0.05$.

Physico-Chemical Properties	COMPOST 1	COMPOST 2
pH_{H_2O}	$9.05^{b} \pm 0.1$ very strongly alkaline	$9.90^{a} \pm 0.1$ very strongly alkaline
pH_{KCl}	$8.39^{b} \pm 0.1$	$9.28^{a} \pm 0.1$
E.C.	$5.01^{a} \pm 0.12$	$5.06^{a} \pm 0.11$
Water content	$56.8^{a} \pm 2$	$45.9^{b} \pm 1.5$
TOC	$16.8^{b} \pm 0.9$	$24.0^{a} \pm 1$
TN (%)	$0.78^{b} \pm 0.05$	$2.0^{a} \pm 0.1$
C/N	$21.57^{a} \pm 1$	$11.97^{b} \pm 0.9$
NH_4^+-N/NO_3-N	$1.30^{b} \pm 0.3$	$2.80^{a} \pm 0.2$
ON/TN	$90^{a} \pm 2$	$60^{b} \pm 1$
WSP	$0.90^{b} \pm 0.05$	$7.03^{a} \pm 0.3$



(**a**) (**b**)

Figure 1. Cation concentration (mgL^{-1}) (**a**) and anion concentration (mgL^{-1}) (**b**) detected in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process at the end of the composting process. Different letters indicate significant differences (Turkey's test $p \leq 0.05$).

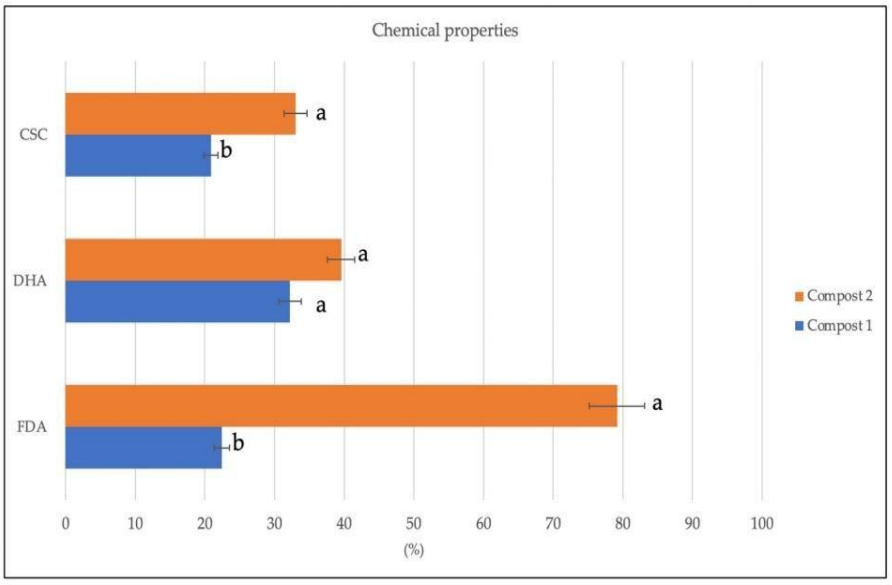


Figure 2. Fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.w.), dehydrogenase (DHA, µg TTF g^{-1} h^{-1}d.w.), cation exchange capacity (CEC, $cmol^{(+)}$ Kg^{-1}) detected in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process. Different letters indicate significant differences (Turkey's test $p \leq 0.05$).

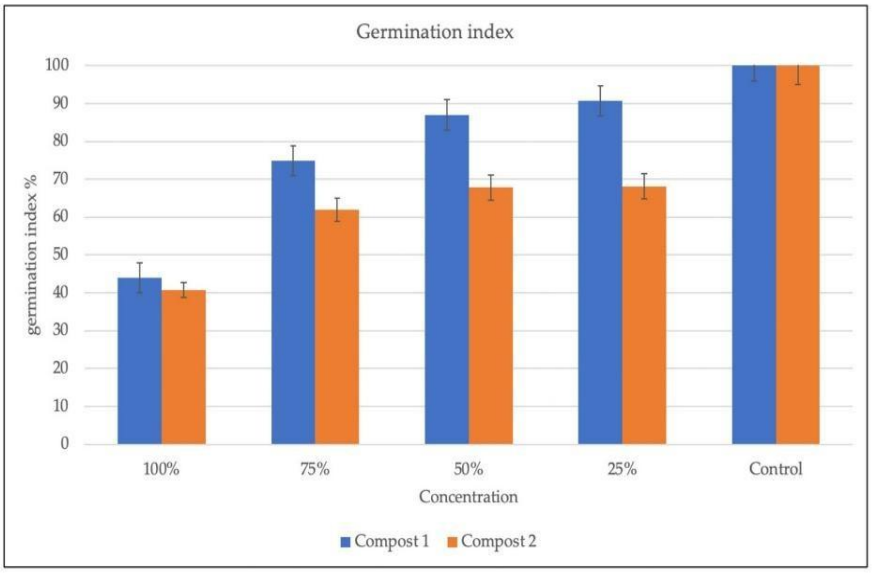


Figure 3. Germination index in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process.

In terms of adsorption capacity for composts, it was observed that all the composts exhibited the ability to adsorb both sodium and chloride, albeit to varying degrees. The phenomenon of negative values recorded at 0 mM of sodium and chloride can be explained by considering that, in the absence of the addition of these two ions in the solutions, other ions remain adsorbed in the compost-available surfaces. This process leads to the generation of negative adsorption values for sodium and chloride. Notably, C2 demonstrated the

highest sodium adsorption capacity, outperforming the other compost. Meanwhile, C1 exhibited an optimal sodium removal capacity at 50 mM NaCl, with a subsequent decline in efficiency as the sodium concentration increased (Table 3). Turning attention to chloride adsorption capacity, it was observed that all composts possessed the capability to remove chloride ions. As the chloride concentration increased, the adsorption capacity of all composts gradually intensified. Notably, C2 displayed the most significant adsorption capacity for chloride ions, further emphasizing its efficacy in the removal of both sodium and chloride.

Table 3. The data regarding the absorption capacity of the analyzed compost related to sodium and chloride. Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

	0 mM	25 mM	50 mM	100 mM	150 mM
ID	Na^+	Na^+	Na^+	Na^+	Na^+
C1	$-4.56^{e} \pm 0.15$	$8.09^{d} \pm 0.76$	$12.60^{c} \pm 0.23$	$53.58^{b} \pm 0.24$	$93.95^{a} \pm 1.4$
C2	$-3.26^{e} \pm 0.2$	$88.11^{d} \pm 0.6$	$109.51^{c} \pm 0.2$	$243.50^{a} \pm 0.4$	$212.88^{b} \pm 1.3$
ID	Cl^-	Cl^-	Cl^-	Cl^-	Cl^-
C1	$-5.86^{e} \pm 0.2$	$53.23^{d} \pm 0.16$	$120.59^{c} \pm 1.6$	$138.23^{b} \pm 1.8$	$367.99^{a} \pm 3.6$
C2	$-4.330^{e} \pm 0.1$	$87.40^{d} \pm 0.5$	$124.08^{c} \pm 0.1$	$311.51^{b} \pm 0.1$	$461.24^{a} \pm 0.2$

3.2. Soil Characteristics

Table 4 shows the analysis of the soil at time zero, before initiating the various fertilization treatments. It was an alkaline sandy-loam soil, with 2.37% organic matter, poor in anions and cations, and with a CEC of 13 $cmol^{(+)}\ kg^{-1}$. Bacteria were more abundant than fungi and actinomycetes, as also evidenced by a greater DHA with respect to FDA.

Table 4. Chemical and biochemical properties of soil located in Motta San Giovanni before fertilization. WC (water content %), pH $_{H_2O}$ in water and pH_{KCl} in potassium chloride; EC = electric conductivity (dS/m); WSP = water-soluble phenols (µg TAE g^{-1} ds): OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon–nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (µg C g^{-1} f.s.); Dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.s.), fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.s.), BACT (bacteria, UFC g^{-1} f.s.), FUN (fungi (UFC g^{-1} f.s.), ACT (Actinomycetes, UFC g^{-1} f.s.), CEC = cation exchange capacity ($cmol^{(+)}\ Kg^{-1}$ d.s.). Data are the means of three replicates ± standard deviation.

	SOIL
Skeleton (%)	45 ± 0.01
Sandy %	65 ± 0.02
Clay %	23 ± 0.12
Silt %	12 ± 0.23
Textural Class	Sandy-loam
WC	18 ± 0.4
pH (H_2O)	8.5 ± 0.32
pH (KCl)	7.8 ± 0.53
EC	307.3 ± 12.3
CEC ($cmol^{(+)}\ kg^{-1}$)	16 ± 1.7
OC	1.37 ± 0.13
TN	0.19 ± 0.14
C/N	7.21 ± 0.13
WSP	276.1 ± 4.5
MBC	376 ± 8.6

Table 4. *Cont.*

	SOIL
FDA	2.1 ± 0.12
DHA	15.11 ± 0.22
BACT	0.9×10^5
FUN	2.6×10^4
ACT	2.7×10^4
Na^+	0.117 ± 0.32
K^+	0.100 ± 0.26
Ca^{2+}	0.311 ± 0.06
Mg^{2+}	0.011 ± 0.16
Cl^-	0.222 ± 0.11
NO_2^-	nd
NO_3^-	nd
PO_4^{3-}	nd
SO_4^{2-}	0.134 ± 0.11

All the fertilizers used (both composts, NPK, and HM) affected the soil's chemical properties with respect to the control, except for texture, which remained unchanged.

The pH did not change with the treatments. Instead, the EC increased in a particular way with the additions of both composts and much more with C2, suggesting an addition of nutrients. Adding composts to the soil can provide a great quantity of nutrients in the form of hydrated salts, helping to increase the percentage of water in the soils.

The Pearson correlation coefficient also evidenced synergies between cations and among cations and anions. Shortly, potassium was correlated with calcium, suggesting a synergism among them and also with anions, in particular with sulfate (Tables 5 and 6).

Table 5. Soil ions and cations correlation matrix. Pearson values in bold are different from 0 with a significance level alpha = 0.05.

Variables	Na^+	K^+	Ca^{2+}	Mg^{2+}	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
Na^+	**1**	**0.908**	0.125	0.326	0.582	**0.998**	**0.998**	**0.998**	**0.931**
K^+	**0.908**	**1**	0.408	0.582	0.759	**0.893**	**0.893**	**0.893**	**0.980**
Ca^{2+}	0.125	0.408	**1**	**0.887**	0.793	0.090	0.090	0.090	0.441
Mg^{2+}	0.326	0.582	**0.887**	**1**	0.672	0.309	0.309	0.309	0.635
Cl^-	0.582	0.759	0.793	0.672	**1**	0.540	0.540	0.540	0.751
NO_2^-	**0.998**	**0.893**	0.090	0.309	0.540	**1**	1.000	**1.000**	**0.921**
NO_3^-	**0.998**	**0.893**	0.090	0.309	0.540	**1.000**	**1**	**1.000**	**0.921**
PO_4^{3-}	**0.998**	**0.893**	0.090	0.309	0.540	**1.000**	**1.000**	**1**	**0.921**
SO_4^{2-}	**0.931**	**0.980**	0.441	0.635	0.751	**0.921**	**0.921**	**0.921**	**1**

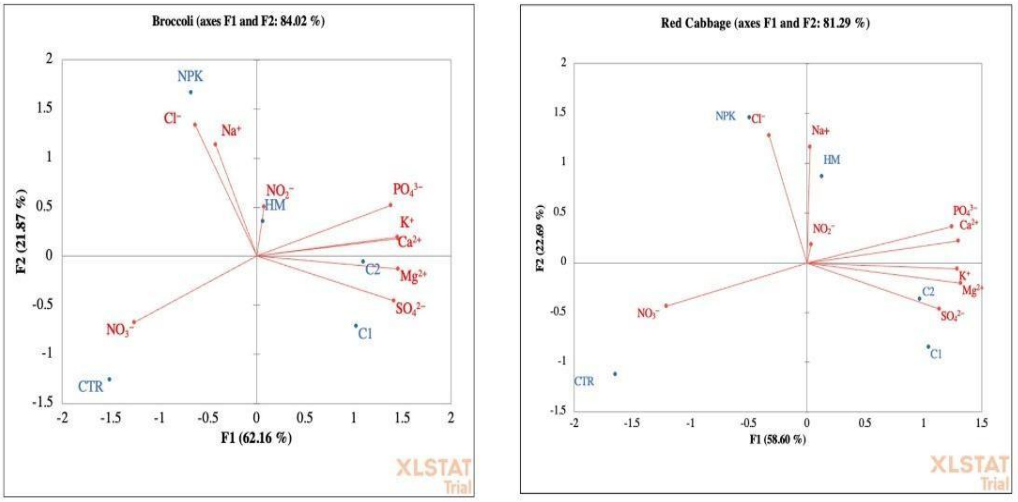
Table 6. Cations and anions concentrations (mg/L) detected 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw + 90% wet wastes Na^+ (sodium), K^+ (potassium), Ca^{2+} (calcium), Mg^{2+} (magnesium), Cl^- (chloride), NO_2^- (nitrite), NO_3^- (nitrate), PO_4^{3-} (phosphate), SO_4^{2-} (sulfate). Data are the means of three replicates ± standard deviation. Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$).

Soil Cations	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2
Na^+	$0.124^{b} \pm 0.02$	$0.119^{b} \pm 0.08$	$0.101^{b} \pm 0.10$	$0.155^{b} \pm 0.09$	$0.91^{a} \pm 0.07$
K^+	$0.116^{c} \pm 0.07$	$0.165^{b} \pm 0.03$	$0.145^{bc} \pm 0.12$	$0.199^{a} \pm 0.11$	$0.290^{a} \pm 0.06$
Ca^{2+}	$0.254^{b} \pm 0.32$	$0.234^{b} \pm 0.22$	$0.346^{b} \pm 0.27$	$0.495^{b} \pm 0.19$	$3.53^{a} \pm 0.16$
Mg^{2+}	$0.019^{a} \pm 0.23$	$0.021^{a} \pm 0.31$	$0.027^{a} \pm 0.12$	$0.029^{a} \pm 0.22$	$0.027^{a} \pm 0.12$
Soil Anions	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2

Table 6. *Cont.*

Soil Cations	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2
Cl^-	0.222 ^{b} ± 0.23	0.206 ^{b} ± 0.34	0.208 ^{b} ± 0.21	0.310 ^{a} ± 0.07	0.298 ^{a} ± 0.02
NO_2^-	nd	nd	nd	nd	0.01 ± 0.01
NO_3^-	nd	nd	nd	nd	0.06 ± 0.02
PO_4^{3-}	nd	nd	nd	nd	0.003 ± 0.01
SO_4^{2-}	0.134 ^{c} ± 0.32	0.339 ^{b} ± 0.12	0.479 ^{b} ± 0.17	0.769 ^{b} ± 0.19	1.65 ^{a} ± 0.18

A PCA analysis demonstrated that C1 and C2 in red cabbage soil correlated with sulfate, magnesium, and potassium. NPK correlated with chloride; CTR with nitrate; and HM with the nitrite, phosphate, calcium, and sodium (Figure 4b). The scenario changed in soil with broccoli. C1 and C2 were both correlated with magnesium and sulfate. HM correlated as for red cabbage with the addition of potassium (Figure 4a).



(**a**) (**b**)

Figure 4. Principal component analyses of ions and cations soil with broccoli (**a**) and red cabbage (**b**). CTR (Control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes.

Organic carbon was the highest with composts. Total nitrogen was the greatest in NPK treatment. The C/N value was higher in soil fertilized with HM and composts with respect to CTR and NPK. WSP was the lowest in compost treatments, while DHA, MBC, bacteria, and actinomycetes were the highest. Fungi and FDA were more abundant in CTR and soil treated with NPK and HM (Table 7). A PCA analysis evidenced a strong positive correlation between C1 MBC, DHA, CEC, OC, and C/N, while C2 correlated better with bacteria, actinomycetes, and OC. HM and NPK were instead correlated with FDA, fungi, and WSP (Figure 5).

Pearson correlation coefficient evidenced a positive, significant similar tendency between organic matter, MBC, CEC, DHA, bacteria, and actinomycetes, suggesting that increasing the SOM amount also increased the amount of microbial biomass as well as the enzymes belonging to the oxo-reductase category, as also demonstrated by the increase in bacteria and actinomycete colonies (Table 8).

Table 7. Chemical and biochemical properties of soil located in Motta San Giovanni, 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes. WC (water content %), pH_{H_2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (dS/m); WSP = water-soluble phenols (µg TAE g^{-1} ds); OC = organic carbon (%); TN = total nitrogen (%); C/N = carbon nitrogen ratio; OM = organic matter (%); MBC = microbial biomass carbon (µg C g^{-1} f.s.); dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.s.), fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.s.), BACT (bacteria, UFC g^{-1} f.s.), FUN (fungi (UFC g^{-1} f.s.), ACT (Actinomycetes, UFC g^{-1} f.s.), CEC = cation exchange capacity ($cmol^{(+)}$ Kg^{-1}d.s.).

Soil Chemical Analyses	CTR	Soil + NPK	Soil + HM	Soil + C1	Soil + C2
WC (%)	$21.4^{b} \pm 0.02$	$22.2^{b} \pm 0.01$	$25.6^{a} \pm 0.03$	$25.2^{a} \pm 0.01$	$25.5^{a} \pm 0.01$
pH (H_2O)	$8.45^{a} \pm 0.12$	$8.46^{a} \pm 0.02$	$8.47^{a} \pm 0.05$	$8.44^{a} \pm 0.05$	$8.41^{a} \pm 0.01$
pH (KCl)	$7.1^{a} \pm 0.07$	$7.01^{a} \pm 0.06$	$6.99^{a} \pm 0.05$	$6.94^{a} \pm 0.04$	$6.97^{a} \pm 0.05$
EC	$350^{c} \pm 0.23$	$301^{c} \pm 0.22$	$297^{c} \pm 0.12$	$530^{b} \pm 0.17$	$740^{a} \pm 0.14$
OC	$1.78^{b} \pm 0.19$	$1.69^{b} \pm 0.22$	$2.13^{ab} \pm 0.11$	$2.9^{a} \pm 0.09$	$3.3^{a} \pm 0.09$
TN	$0.19^{a} \pm 0.17$	$0.23^{a} \pm 0.09$	$0.21^{a} \pm 0.13$	$0.19^{a} \pm 0.12$	$0.20^{a} \pm 0.11$
C/N	$9.4^{b} \pm 0.15$	$7.39^{c} \pm 0.15$	$19.1^{a} \pm 0.16$	$15.2^{a} \pm 0.11$	$16.5^{a} \pm 0.14$
WSP	$282^{b} \pm 0.32$	$320^{a} \pm 0.52$	$315^{a} \pm 0.42$	$138^{c} \pm 1.12$	$170^{c} \pm 0.92$
MBC	$433.3^{c} \pm 0.52$	$733^{b} \pm 0.17$	$798^{b} \pm 0.42$	$897.33^{a} \pm 0.52$	$961.4^{a} \pm 0.32$
FDA	$5.14^{a} \pm 0.44$	$5.44^{a} \pm 0.33$	$5.33^{a} \pm 0.27$	$4.88^{b} \pm 0.36$	$4.81^{b} \pm 0.18$
DHA	$20.1^{b} \pm 0.72$	$22.1^{b} \pm 0.32$	$24.1^{b} \pm 0.42$	$32.92^{a} \pm 0.32$	$38.09^{a} \pm 0.42$
BACT	$1.3 \times 10^{5\,c} \pm 1.42$	$1.1 \times 10^{5\,c} \pm 2.12$	$1.6 \times 10^{5\,c} \pm 3.32$	$5 \times 10^{5\,b} \pm 3.13$	$8.3 \times 10^{5\,a} \pm 2.12$
FUN	$4.6 \times 10^{4\,a} \pm 3.12$	$4.5 \times 10^{4\,a} \pm 1.42$	$4.6 \times 10^{4\,a} \pm 2.62$	$2.7 \times 10^{4\,b} \pm 2.11$	$3 \times 10^{4} \pm 2.02^{b}$
ACT	$5.7 \times 10^{4\,a} \pm 2.12$	$3.7 \times 10^{4\,b} \pm 4.12$	$6.7 \times 10^{4\,a} \pm 1.12$	$1.3 \times 10^{5\,c} \pm 3.16$	$1.5 \times 10^{5\,c} \pm 2.21$
CEC	$16^{b} \pm 0.13$	$12^{c} \pm 0.12$	$19^{ba} \pm 0.18$	$22^{a} \pm 0.11$	$22.9^{a} \pm 0.15$

Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 15) ± standard deviation.

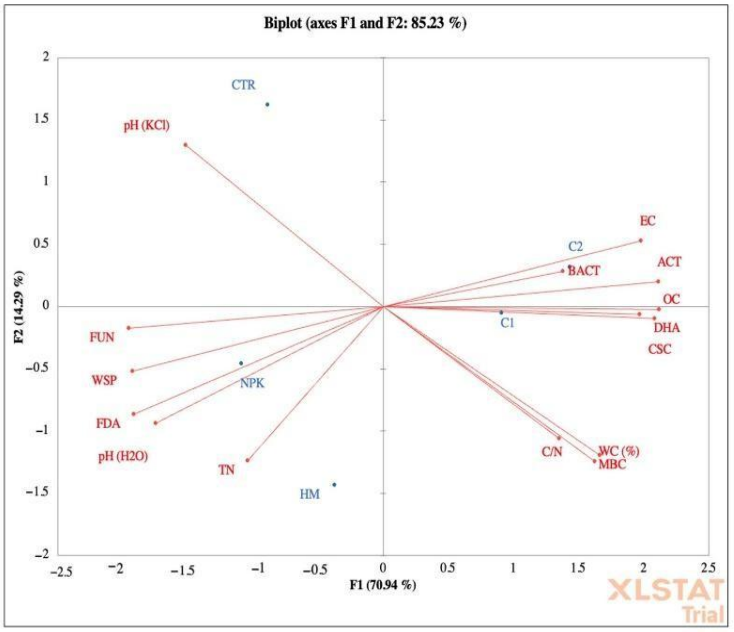


Figure 5. Principal component analyses of chemical and biochemical properties of soil located in Motta San Giovanni before fertilization. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes.

Table 8. Correlation matrix (Pearson) of chemical and biochemical properties of soil located in Motta San Giovanni, 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw and 90% wet wastes. Values in bold are different from 0 with a significance level of alpha = 0.05.

Variables	WC (%)	pH (H_2O)	pH (KCl)	EC	OC	TN	C/N	WSP	MBC	FDA	DHA	BACT	FUN	ACT	CEC
WC (%)	**1**	−0.315	−0.841	0.529	0.770	−0.175	**0.934**	−0.525	0.863	−0.473	0.740	0.403	−0.593	0.714	0.825
pH (H_2O)	−0.315	**1**	0.312	**−0.971**	−0.831	0.441	−0.189	0.794	−0.468	**0.879**	−0.847	−0.815	0.774	−0.848	−0.651
pH (KCl)	−0.841	0.312	**1**	−0.506	−0.715	−0.059	−0.602	0.602	**−0.959**	0.403	−0.752	−0.229	0.736	−0.655	−0.609
EC	0.529	**−0.971**	−0.506	**1**	**0.939**	−0.444	0.390	−0.860	0.641	**−0.912**	**0.947**	0.806	−0.861	**0.942**	0.787
OC	0.770	−0.831	−0.715	**0.939**	**1**	−0.463	0.637	**−0.895**	0.794	**−0.894**	**0.988**	0.677	**−0.913**	**0.992**	**0.921**
TN	−0.175	0.441	−0.059	−0.444	−0.463	**1**	−0.314	0.641	0.100	0.758	−0.341	−0.093	0.466	−0.565	−0.659
C/N	**0.934**	−0.189	−0.602	0.390	0.637	−0.314	**1**	−0.372	0.631	−0.406	0.561	0.351	−0.383	0.598	0.813
WSP	−0.525	0.794	0.602	−0.860	**−0.895**	0.641	−0.372	**1**	−0.591	**0.947**	−0.874	−0.394	**0.977**	**−0.934**	−0.819
MBC	0.863	−0.468	**−0.959**	0.641	0.794	0.100	0.631	−0.591	**1**	−0.456	0.841	0.485	−0.729	0.720	0.648
FDA	−0.473	**0.879**	0.403	**−0.912**	**−0.894**	0.758	−0.406	**0.947**	−0.456	**1**	−0.850	−0.558	**0.879**	**−0.942**	−0.861
DHA	0.740	−0.847	−0.752	**0.947**	**0.988**	−0.341	0.561	−0.874	0.841	−0.850	**1**	0.704	**−0.919**	**0.968**	0.851
BACT	0.403	−0.815	−0.229	0.806	0.677	−0.093	0.351	−0.394	0.485	−0.558	0.704	**1**	−0.416	0.636	0.521
FUN	−0.593	0.774	0.736	−0.861	**−0.913**	0.466	−0.383	**0.977**	−0.729	**0.879**	**−0.919**	−0.416	**1**	**−0.928**	−0.784
ACT	0.714	−0.848	−0.655	**0.942**	**0.992**	−0.565	0.598	**−0.934**	0.720	**−0.942**	**0.968**	0.636	**−0.928**	**1**	**0.932**
CEC	0.825	−0.651	−0.609	0.787	**0.921**	−0.659	0.813	−0.819	0.648	−0.861	0.851	0.521	−0.784	**0.932**	**1**

3.3. Crop Growth Data

In the presence of both composts, red cabbage exhibited a significant augmentation in leaf width, leaf area, leaf length, and plant height compared to the control, NPK, and HM treatments. The fruit-head diameter when C1 and C2 were applied shows an approximate 50% increase compared to the control and a 25% increase compared to HM and NPK. Productivity, measured in tons per hectare, experienced a noteworthy enhancement of 15% compared to NPK and HM in the presence of both composts. Notably, C1 demonstrated the most substantial effect on productivity, boasting a 35% increase compared to the control and a 30% increase compared to HM and NPK (Table 9).

Table 9. Growth parameters and productivity (tons per hectare) of red cabbage and broccoli grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes.

Red Cabbage	CTR	NPK	HM	C1	C2
Leaf humidity (%)	$84^{a} \pm 0.11$	$84^{a} \pm 0.62$	$86^{a} \pm 0.42$	$86^{a} \pm 0.32$	$85^{a} \pm 0.46$
Leaf width (cm)	$5.7^{a} \pm 0.56$	$8.8^{ab} \pm 0.25$	$1^{a} \pm 0.42$	$14^{a} \pm 0.15$	$13^{a} \pm 0.52$
Leaf length (cm)	$4.4^{a} \pm 0.12$	$7.8^{ab} \pm 0.41$	$10^{a} \pm 0.68$	$9.5^{a} \pm 0.42$	$10^{a} \pm 0.23$
Leaf area (cm^2)	$45^{c} \pm 0.25$	$65^{b} \pm 0.42$	$75^{b} \pm 0.13$	$96^{a} \pm 0.32$	$91^{a} \pm 0.15$
Plant height (cm)	$20^{c} \pm 0.125$	$30^{b} \pm 0.14$	$35^{b} \pm 0.12$	$43^{a} \pm 0.12$	$40^{a} \pm 0.43$
Head diameter (cm)	$10^{b} \pm 1.42$	$12^{a} \pm 2.32$	$12^{a} \pm 1.72$	$15^{a} \pm 2.52$	$15^{a} \pm 1.52$
Yield (Tons/ha)	$36^{b} \pm 1.51$	$42^{a} \pm 1.42$	$42^{a} \pm 1.32$	$49^{a} \pm 2.12$	$47^{a} \pm 2.32$

Table 9. *Cont.*

Red Cabbage	CTR	NPK	HM	C1	C2
Broccoli Calabrese	CTR	NPK	HM	C1	C2
Leaf humidity (%)	$84^{a} \pm 0.15$	$84^{a} \pm 0.18$	$86^{a} \pm 0.62$	$86^{a} \pm 0.43$	$85^{a} \pm 0.65$
Leaf width (cm)	$9^{a} \pm 3.32$	$12^{a} \pm 3.44$	$11^{a} \pm 2.32$	$15^{a} \pm 2.23$	$14^{a} \pm 1.12$
Leaf length (cm)	$14^{a} \pm 2.42$	$17^{a} \pm 3.22$	$18^{a} \pm 2.12$	$18^{a} \pm 0.22$	$18^{a} \pm 0.16$
Leaf area (cm^2)	$70^{b} \pm 0.29$	$165^{a} \pm 0.59$	$175^{a} \pm 0.54$	$196^{a} \pm 0.44$	$191^{a} \pm 0.12$
Plant height (cm)	$50^{b} \pm 0.34$	$60^{b} \pm 0.14$	$65^{ab} \pm 2.42$	$80^{a} \pm 2.12$	$75^{a} \pm 2.32$
Head diameter (cm)	$10^{b} \pm 2.12$	$16^{a} \pm 2.32$	$15^{a} \pm 3.10$	$19^{a} \pm 3.11$	$19^{a} \pm 3.12$
Yield (Tons/ha)	$5^{c} \pm 3.12$	$15^{b} \pm 4.01$	$19^{ab} \pm 2.12$	$22^{a} \pm 4.2$	$21^{a} \pm 3.11$

Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 15) ± standard deviation.

Similarly, broccoli calabrese, when exposed to C1 and C2, exhibited a significant surge in growth. The leaf area tripled in comparison to the control, surpassing NPK and HM by 20%. Productivity, experiencing a fourfold increase compared to the control, surpassed NPK by 40% and HM by 15% (Table 9).

Chlorophyll (Table 10) data evidenced a greater amount of total chlorophyll and Cha/Chb ratio in broccoli and red cabbage treated with C1 and C2 with respect to CTR, HM, and NPK. Regarding the photosynthetic parameters of chlorophyll fluorescence, Fv, Fm, F0, and Y(NPQ) were the lowest in broccoli and red cabbage treated with both composts. Conversely, the Fv/Fm ratio, Y(NO), and ETR were instead the highest both in broccoli and red cabbage treated with both composts.

Table 10. Content of chlorophyll a (Chl a, mg 100 g^{-1}FW), chlorophyll b (Chl b, mg 100 g^{-1}FW), total chlorophyll (TChl, mg 100 g^{-1}FW), chlorophyll a/chlorophyll b (Chl a/Chl b) and photosynthetic parameters (FV, Fm, Y(NPQ), Y(NO) and ETR are expressed as µmol m^{-2} s^{-1}) in leaves of bed cabbage and broccoli calabrese.

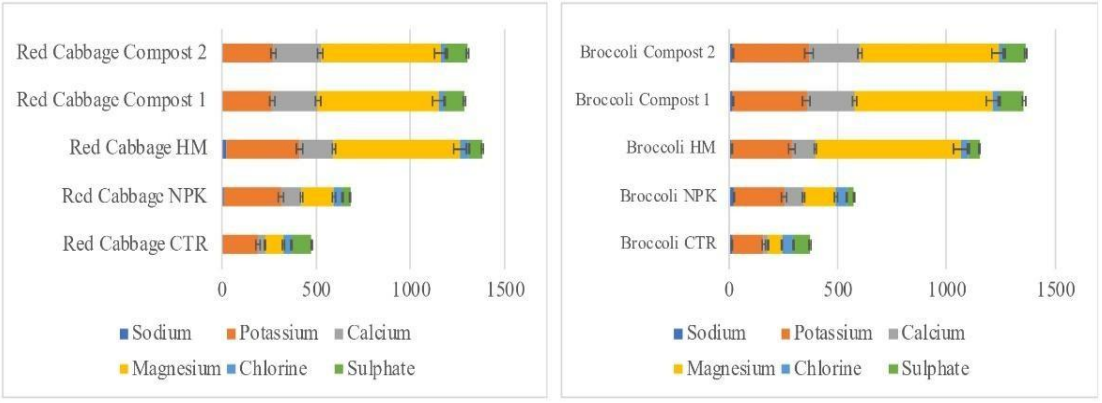
Broccoli Calabrese	CTR	NPK	HM	C1	C2
Chl a	$114^{b} \pm 0.43$	$120^{b} \pm 0.12$	$142^{a} \pm 0.02$	$158^{a} \pm 0.12$	$167^{a} \pm 0.19$
Chl b	$60^{a} \pm 2.25$	$54^{a} \pm 3.11$	$55^{a} \pm 1.11$	$57^{a} \pm 1.45$	$59^{a} \pm 1.24$
Chla/Chlb	$1.9^{b} \pm 1.11$	$2.2^{b} \pm 1.12$	$2.58^{a} \pm 1.54$	$2.77^{a} \pm 1.12$	$2.83^{a} \pm 1.02$
T Chl	$174^{b} \pm 5.12$	$174^{b} \pm 4.12$	$197^{ab} \pm 3.14$	$215^{a} \pm 4.11$	$226^{a} \pm 2.12$
FV	$0.621^{b} \pm 0.43$	$0.802^{ab} \pm 0.22$	$1.004^{a} \pm 0.12$	$1.007^{a} \pm 0.52$	$1.107^{a} \pm 0.23$
Fm	$0.939^{a} \pm 0.65$	$1.077^{a} \pm 0.21$	$1.423^{a} \pm 0.22$	$1.222^{a} \pm 0.61$	$1.343^{a} \pm 0.36$
F0	$0.293^{b} \pm 0.02$	$0.384^{ab} \pm 0.02$	$0.528^{a} \pm 0.11$	$0.534^{a} \pm 0.12$	$0.544^{a} \pm 0.74$
Fv/Fm	$0.661^{a} \pm 0.02$	$0.74^{a} \pm 0.01$	$0.71^{a} \pm 0.12$	$0.82^{a} \pm 0.01$	$0.82^{a} \pm 0.01$
Y(NPQ)	$0.443^{a} \pm 0.01$	$0.329^{a} \pm 0.11$	$0.216^{a} \pm 0.02$	$0.215^{a} \pm 0.04$	$0.219^{a} \pm 0.01$
Y(NO)	$0.235^{b} \pm 0.02$	$0.215^{b} \pm 0.01$	$0.344^{a} \pm 0.01$	$0.397^{a} \pm 0.03$	$0.361^{a} \pm 0.04$
ETR	$21.21^{c} \pm 0.12$	$28.84^{b} \pm 0.16$	$35.24^{b} \pm 0.14$	$41.26^{a} \pm 0.13$	$39.54^{a} \pm 0.14$

Table 10. *Cont.*

Broccoli Calabrese	CTR	NPK	HM	C1	C2
Red Cabbage	CTR	NPK	HM	C1	C2
Chl a	94 ^{a} ± 0.56	100 a ± 1.52	112 ^{a} ± 4.12	118 ^{a} ± 4.67	117 ^{a} ± 5.12
Chl b	65 ^{a} ± 3.52	69 ^{a} ± 3.15	66 ^{a} ± 2.17	65 ^{a} ± 2.15	69 ^{a} ± 1.21
Chla/Chlb	1.45 ^{a} ± 0.01	1.45 ^{a} ± 0.42	1.47 ^{a} ± 0.13	1.81 ^{a} ± 0.13	1.71 ^{a} ± 0.11
T Chl	159 ^{b} ± 8.76	169 ^{a} ± 8.22	178 ^{a} ± 4.62	183 ^{a} ± 2.24	186 ^{a} ± 4.12
FV	0.644 ^{b} ± 0.02	0.776 ^{b} ± 0.01	1.016 ^{a} ± 0.01	1.027 ^{a} ± 0.02	1.144 ^{a} ± 0.02
Fm	0.899 ^{b} ± 0.03	1.000 ^{b} ± 0.25	1.227 ^{a} ± 0.26	1.392 ^{a} ± 0.11	1.465 ^{a} ± 0.12
F0	0.293 ^{b} ± 0.05	0.384 ^{b} ± 0.06	0.528 ^{a} ± 0.08	0.534 ^{a} ± 0.04	0.544 ^{a} ± 0.06
Fv/Fm	0.617 ^{a} ± 0.01	0.626 ^{a} ± 0.05	0.639 ^{a} ± 0.03	0.656 ^{a} ± 0.04	0.663 ^{a} ± 0.01
Y(NPQ)	0.433 ^{a} ± 0.02	0.409 ^{a} ± 0.08	0.216 ^{b} ± 0.03	0.225 ^{b} ± 0.07	0.256 ^{b} ± 0.09
Y(NO)	0.235 ^{b} ± 0.07	0.215 ^{b} ± 0.03	0.344 ^{a} ± 0.05	0.397 ^{a} ± 0.03	0.361 ^{a} ± 0.05
ETR	21.21 ^{c} ± 1.03	28.84 ^{b} ± 3.12	35.24 ^{b} ± 2.12	41.26 ^{a} ± 4.02	39.54 ^{a} ± 3.02

Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 15) ± standard deviation.

The ions were predominantly present in red cabbage and broccoli treated with both composts. Magnesium, calcium, and potassium were the most abundant cations in both crop species treated with composts C1 and C2 (Figure 6).



(**a**) (**b**)

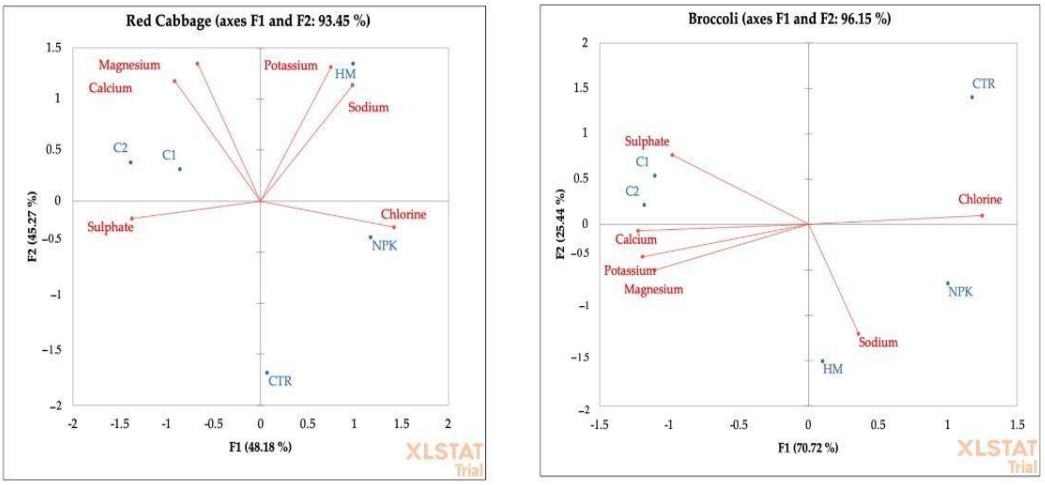
Figure 6. Bioaccumulation factor of red cabbage (**a**) and broccoli (**b**) grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes. Values are expressed as micrograms and are the mean of three replicates (n = 15) with errors standard.

Considering the bioaccumulation factor, red cabbage grown with composts 1 and 2 accumulated more magnesium, calcium, and sulfate in its leaves with respect to the other treatments. Similar results were observed for broccoli, with a greater increase even in potassium compared with the other treatments.

The best accumulation of ions has been observed in broccoli leaves treated with both composts (Figure 6).

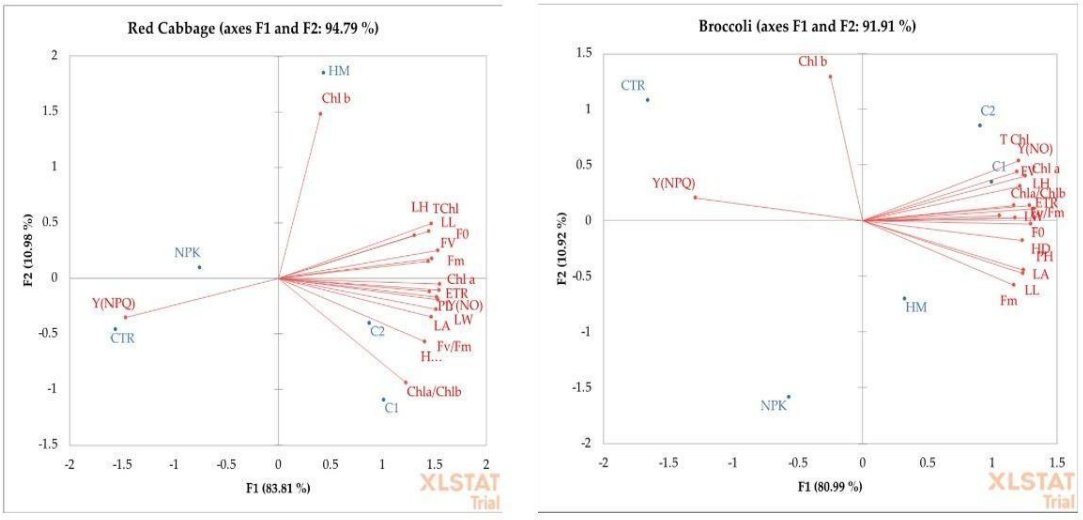
From the PCA, it emerged that broccoli cultivated with both composts accumulated sulfates, instead of HM, NPK, more sodium, and CTR chloride. (Figure 7b). The PCA related to red cabbage bioaccumulation factors evidenced an accumulation of magnesium and calcium with both composts; NPK and CTR showed an accumulation of Cl and HM of Na and K. (Figure 7a). Chlorophyll a and the photosynthesis parameters (ETR, Fm/Fv, and Y(NO) were mostly expressed in the presence of both composts in both crops. HM

correlated with total chlorophyll, chlorophyll B, F0, Fm, and Fv. No correlation between NPK and the parameters linked to photosynthesis activity has been found (Figure 8).



(**a**) (**b**)

Figure 7. PCA of ions and cations of red cabbage (**a**) and broccoli (**b**) grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes.



(**a**) (**b**)

Figure 8. Principal component analyses of the content of chlorophyll a (Chl a, mg 100 g^{-1}FW), chlorophyll b (Chl b, mg 100 g^{-1}FW), total chlorophyll (TChl, mg 100 g^{-1}FW), chlorophyll a/chlorophyll b (Chl a/Chl b) and photosynthetic parameters (FV, Fm, Y(NPQ), Y(NO), and ETR are expressed as µmol m^{-2} s^{-1}), in leaves of red cabbage (**a**) and broccoli (**b**) grown in not-amended soil (control, CTR), NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% Straw + 90% wet wastes.

4. Discussion

4.1. Compost Effects on Soil Chemical and Biological Properties

Composts 1 and 2 significantly affected soil properties, increasing EC and significantly enhancing organic matter content, CEC, microbial biomass, bacteria, and actinomycetes. The observed decrease in phenol content in compost-treated soils compared to the other treatments suggested that composts may have an impact on soil microorganisms and their metabolic activities. In fact, an increase in bacteria and actinomycetes has been observed as well. The observed significant increase in actinomycetes is of paramount importance due to their critical role in the cycling of organic matter. Actinomycetes serve as a natural barrier against a wide array of plant pathogens within the rhizosphere, effectively suppressing their growth. Moreover, they are adept at breaking down complex polymer mixtures present in deceased plants, animals, and fungi. This breakdown process facilitates the production of a diverse array of extracellular enzymes, which have been shown to significantly benefit crop production, enhancing both yield and health [36–38].

Further expanding on their beneficial impact, it has been demonstrated that actinomycetes not only augment the levels of nutrients and organic matter in the soil but also substantially increase the soil's microbial biomass [39]. This, in turn, boosts nitrogen availability, a critical component for plant growth, by stimulating the activity of essential nitrogen-metabolizing enzymes. The enhancement of nitrogen availability is particularly noteworthy, as it directly supports the growth and productivity of crops.

The multifaceted benefits of actinomycetes, from pathogen suppression and organic matter decomposition to nutrient enhancement and nitrogen availability, underscore their invaluable contribution to sustainable agriculture. By leveraging the positive roles of actinomycetes, it is possible to advance sustainable food-production practices that are both productive and environmentally friendly. This approach not only aims at achieving higher crop yields but also emphasizes biosafety and the preservation of ecological balance, making both composts a cornerstone in the pursuit of global food security and sustainable agricultural development.

The results are corroborated by the Pearson coefficient data, which reveal a positive correlation among microbial biomass carbon (MBC), organic content, water content (WC), dehydrogenase activity (DHA), and actinomycete populations. Such correlations were observed in soil samples collected from areas cultivating red cabbage and broccoli, where both types of compost were applied. These observations highlight the intricate interplay between soil characteristics and the microbial shifts following fertilizer application. The findings align with the research conducted by Arunrat et al. (2023) [40], which demonstrated that consistent fertilizer use and tillage methods over five years markedly enhanced the diversity and abundance of soil microbial communities.

The study reveals that both bacterial and actinomycete populations were significantly affected by Compost 2, as demonstrated by a PCA analysis (Figure 5). In contrast, Compost 1 was found to have a positive correlation with microbial biomass, water content, cation exchange capacity (CEC), and dehydrogenase activity (DHA). These findings indicate that are the characteristics of each type of compost that, influencing specific soil parameters, enhance or modify soil ecosystem functions.

This research suggests that it is not the inherent soil properties that remained consistent across different crops in this study, nor is it the type of crop cultivated that primarily influences soil ecosystem functioning. Instead, the key factor appears to be the raw material chosen for compost production. During the composting process, these raw materials are transformed into various bio-compounds, each possessing distinct specificities that can lead to different effects on the soil ecosystem.

In essence, the study highlights the critical role of compost composition in shaping soil health and functionality. By selecting appropriate compost materials, it is possible to tailor soil conditions to support desired ecosystem functions, thereby optimizing agricultural productivity and sustainability.

Notable changes in enzyme activities have been also observed. However, the correlation pattern between MBC and DHA with the addition of both composts evidenced the impact of composts on the soil's oxidative processes. It is important to note that both HM and NPK failed to exhibit a significant association with the chemical and biochemical attributes related to soil fertility.

In summary, these results offer crucial insights into the complex interplay among fertilizers, soil properties, and microbial interactions, which are fundamental for developing knowledgeable soil-management strategies and promoting sustainable agricultural practices. By closely monitoring these variables, we can evaluate soil health, microbial function, and nutrient cycling within variously treated soil ecosystems, thereby enhancing environmental stewardship. This approach not only aids in optimizing agricultural output but also in preserving ecological balance, ensuring a sustainable future for farming practices.

4.2. Compost Effects on Crop Yield and Quality

The changes noted had a beneficial impact on the yield and quality of red cabbage and broccoli. It was found that both yield and quality were linked to the levels of organic matter in the soil, a key factor in soil fertility and functionality. Organic matter contains trace elements vital for the needs of soil microorganisms, enhancing microbial activities. This, in turn, influences the interactions among soil microorganisms, which indirectly affects crop productivity. Such dynamics underscore the critical role of organic matter in supporting agricultural success, highlighting its importance in both soil health and crop performance.

The differences in both crops, grown with both composts, compared to the control and the other fertilizers were more evident in parameters related to leaf area, width, and length as well as head diameter. These results were supported by photosynthesis parameters and pigments that were increased in compost-treated crops than in the control and the NPK- and HM-treated crops. Total chlorophyll increased in crops grown with composts, probably because it correlated to the greatest leaf area. The method of chlorophyll fluorometry offers significant insights into the health of photosynthetic systems in plants by measuring the variable fluorescence of photosystem II [41]. Among the photosynthetic parameters, the ratio of variable fluorescence (Fv) to maximal fluorescence (Fm), known as Fv/Fm, serves as the most commonly utilized indicator. This ratio reflects the efficiency of primary light-energy conversion and the maximal efficiency of photosystem II (PSII) photochemistry [42,43]. The presence of negative effects on plants of external inputs is indicated by a reduced number of open reaction centers, leading to a decreased Fv/Fm ratio [44,45]. In this study, the lowest Fv/Fm values were observed in the control of both crops and in both crops grown with NPK and HM, indicating significant positive effects of composts on their photosynthetic efficiency. Y(II) serves as a metric for assessing plant efficiency, denoting the amount of energy utilized by photosystem II (PSII) under consistent photosynthetic lighting conditions, and is directly linked to the electron transport rate (ETR) and the plant's ability to assimilate carbon [46]. This relationship highlights the critical role of Y(II) in understanding the dynamics of photosynthesis, particularly in how efficiently a plant can convert light energy into chemical energy through PSII, further influencing its growth and productivity by affecting carbon assimilation processes. In the PCA (principal component analysis) of broccoli and red cabbage diagrams, the positioning of C1 and C2 in the right quadrants highlights the particular efficiency of composts on these cultivars. The spatial arrangement in the diagrams clearly illustrates how much weight they have on photosynthetic efficiency and, consequently, on crop growth and productivity. NPQ, which stands for non-photochemical quenching, acts as a measure of how plants dissipate excess light energy as heat within the antenna system to prevent photodamage. It is deemed a crucial short-term photoprotective mechanism in higher plants. With composts in both crops, NPQ values were observed to decrease across all cultivars, while increasing in the control and NPK-treated crops. This suggests that NPK may be the cause of oxidative damage to the photosynthetic apparatus of both crops. This interpretation is supported by the total chlorophyll content (TChl) data, which were the

lowest in the NPK- and HM-treated crops and in the controls of both crops. Crops treated with composts exhibited enhanced ion uptake, a finding substantiated by bioaccumulation-factor data, which indicated that these plants accumulated essential mineral nutrients critical for human health, including magnesium (Mg), calcium, potassium, and sulfate. Current food-supply statistics indicate that approximately half of the global population is at risk of dietary deficiencies in calcium (Ca) and Mg, with this figure escalating to over 95% in 16 African countries. The strategy of biofortifying crops with Mg and Ca has been recommended as a means to bolster dietary intakes for humans [47] as well as livestock [48,49], enhancing overall food-system nutrition. Despite their potential benefits, such biofortification practices have not yet been broadly implemented within agricultural production systems. In summary, both composts evidenced a positive effect on the crop quality of broccoli and red cabbage in comparison to horse manure and synthetic fertilizers.

5. Conclusions

In short, this study has successfully identified and evaluated environmentally friendly technologies for transforming organic wastes into fertilizers, aiming to enhance soil sustainability and crop yields. By comparing two compost formulations (Compost 1 and Compost 2) with horse manure (HM) and synthetic NPK fertilizers, and utilizing unfertilized soil as a control, the research provides compelling evidence on the efficacy of these organic amendments. Despite Compost 1 having a lower organic carbon content and enzyme activity compared to Compost 2, it emerged as the superior soil improver. It significantly increased the labile fraction of organic matter, the activity of oxidative enzymes, microbial biomass, and, importantly, crop yield. These findings underscore the potential of using specific compost formulations, particularly those with a high C/N ratio and effective humification of wet materials, as viable alternatives to conventional fertilizers. This approach not only promises to improve soil health and productivity but also contributes to the broader goal of sustainable agriculture by recycling organic wastes into valuable soil amendments.

Author Contributions: Conceptualization, A.M. (Adele Muscolo) and A.M. (Angela Maffia); methodology, F.M., M.O., C.M. and A.M. (Angela Maffia); software, A.M. (Angela Maffia) and S.B.; validation, A.M. (Adele Muscolo) and C.M.; formal analysis, F.M.; investigation, M.O.; data curation, C.M.; writing—original draft preparation, A.M. (Adele Muscolo); writing—review and editing, A.M. (Adele Muscolo) and A.M. (Angela Maffia); visualization, A.M. (Adele Muscolo); project administration, A.M. (Adele Muscolo); funding acquisition, A.M. (Adele Muscolo). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Calabrian Region, grant number DDL n° 16315 del 13-12-2022, POR CALABRIA FESR-FSE 2014–2020 ASSE I—PROMOZIONE DELLA RICERCA E DELL'INNOVAZIONE.

Data Availability Statement: Suggested data availability statements are available at "https://iris.unirc.it/" (accessed on 8 April 2024).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Muhie, S.H. Novel approaches and practices to sustainable agriculture. *J. Agric. Food Res.* **2022**, *10*, 100446. [CrossRef]
2. Alexandratos, N.; Bruinsma, J. *World Agriculture towards 2030/2050: The 2012 Revision*; ESA Working Papers 12-03; Food and Agriculture Organization of the United Nations (FAO): Rome, Italy, 2012.
3. Willett, W.C.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S.J.; Garnett, T.; Tilman, D.; DeClerck, F.; Wood, A.; et al. Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* **2019**, *393*, 447–492. [CrossRef]
4. Hassan, N.; Wahed, N.H.A.E.; Abdelhamid, A.O.; Ashraf, M.; Abdelfattah, E.A. Composting: An eco-friendly solution for organic waste management to mitigate the effects of climate change. *Innovare J. Soc. Sci.* **2023**, *11*, 1–7. [CrossRef]
5. EU. Commission Implementing Regulation (EU) 2021/1165 Authorising Certain Products and Substances for Use in Organic Production and Establishing Their Lists. 2021. Available online: https://eur-lex.europa.eu/eli/reg_impl/2021/1165/oj (accessed on 15 July 2021).

6. Muscolo, A.; Papalia, T.; Settineri, G.; Mallamaci, C.; Jeske-Kaczanowska, A. Are raw materials or composting conditions and time that most influence the maturity and/or quality of composts? Comparison of obtained composts on soil properties. *J. Clean. Prod.* **2018**, *195*, 93–101. [CrossRef]
7. Goldan, E.; Nedeff, V.; Bârsan, N.; Culea, M.; Panainte-Lehăduș, M.; Mosneguțu, E.; Tomozei, C.; Chițimuș, D.; Irimia, O. Assessment of Manure Compost Used as Soil Amendment—A review. *Processes* **2023**, *11*, 1167. [CrossRef]
8. Ghimire, S.; Chhetri, B.P.; Shrestha, J. Efficacy of different organic and inorganic nutrient sources on the growth and yield of bitter gourd (*Momordica charantia* L.). *Heliyon* **2023**, *9*, e22135. [CrossRef] [PubMed]
9. Bonanomi, G.; De Filippis, F.; Zotti, M.; Idbella, M.; Cesarano, G.; Al-Rowaily, S.L.; Abd-ElGawad, A.M. Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield. *Appl. Soil Ecol.* **2020**, *156*, 103714. [CrossRef]
10. Suman, J.; Rakshit, A.; Ogireddy, S.D.; Singh, S.; Gupta, C.; Chandrakala, J. Microbiome as a key player in sustainable agriculture and human health. *Front. Soil Sci.* **2022**, *2*, 821589. [CrossRef]
11. Insam, H.; Merschak, P. Nitrogen leaching from forest soil cores after amending organic recycling products and fertilizers. *Waste Manag. Res.* **1997**, *15*, 277–291. [CrossRef]
12. Mallakpour, S.; Sirous, F.; Hussain, C.M. Sawdust, a versatile, inexpensive, readily available bio-waste: From mother earth to valuable materials for sustainable remediation technologies. *Adv. Colloid Interface Sci.* **2021**, *295*, 102492. [CrossRef]
13. Ayilara, M.S.; Olanrewaju, O.S.; Babalola, O.O.; Odeyemi, O. Waste Management through Composting: Challenges and Potentials. *Sustainability* **2020**, *12*, 4456. [CrossRef]
14. Nemet, F.; Perić, K.; Lončarić, Z. Microbiological activities in the composting process: A review. *Columella* **2021**, *8*, 41–53. [CrossRef]
15. Liang, C.; Das, K.C.; McClendon, R.W. The Influence of Temperature and Moisture Contents Regimes on the Aerobic Microbial Activity of a Biosolids Composting Blend. *Bioresour. Technol.* **2003**, *86*, 131–137. [CrossRef] [PubMed]
16. ANPA-National Agency for Environmental Protection Guidelines. *"Methods of Compost Analysis", Manuals and Guidelines 3/2001*; 6334 manuali 3; SPED S.r.l.: Roma, Italy, 2001; ISBN 88-448-0258-9.
17. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951. [CrossRef]
18. Perucci, P. Enzyme activity and microbial biomass in a field soil amended with municipal 460 refuse. *Biol. Fertil. Soils* **1992**, *14*, 54–60. [CrossRef]
19. Von Mersi, W.; Schinner, F. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
20. Box, J.D. Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
21. Hendershot, W.H.; Duquette, M. A Simple Barium Chloride Method for Determining Cation Exchange Capacity and Exchangeable Cations. *Soil Sci. Soc. Am. J.* **1986**, *50*, 605–608. [CrossRef]
22. Gariglio, N.; Buyatti, M.; Pilatti, R.A.; Russia, D.E.G.; Acosta, M.R. Use of a germination bioassay to test compost maturity of willow (*Salix* sp.) sawdust. *N. Z. J. Crop Hortic. Sci.* **2002**, *30*, 135–139. [CrossRef]
23. Zucconi, F.; Pera, A.; Forte, M.; De Bertoldi, M. Evaluating toxicity of immature compost. *BioCycle* **1981**, *22*, 54–57.
24. IUSS Working Group WRB. World Reference Base for Soil Resources. In *International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*, 4th ed.; International Union of Soil Sciences (IUSS): Vienna, Austria, 2022.
25. Bouyoucos, G.J. Hydrometer method improved for making particle size analysis of soils. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
26. Muscolo, A.; Settineri, G.; Papalia, T.; Attinà, E.; Basile, C.; Panuccio, M.R. Anaerobic co-digestion of recalcitrant agricultural wastes: Characterizing of biochemical parameters of digestate and its impacts on soil ecosystem. *Sci. Total Environ.* **2017**, *586*, 746–752. [CrossRef]
27. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
28. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoff in organishen Kopern. *Anal. Chem.* **1883**, *22*, 354–358.
29. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
30. Insam, H.; Goberna, M. *Section 4 Update: Use of Biolog® for the Community Level Physiological Profiling (CLPP) of Environmental Samples*; Springer eBooks: Berlin/Heidelberg, Germany, 2008; pp. 2755–2762. [CrossRef]
31. Waksman, S.A. *Soil Microbiology*; John Wiley & Sons: New York, NY, USA, 1952.
32. Johnson, L.F.; Curl, E.A. *Methods for the Research on Ecology of Soil-Borne Plant Pathogens*; Burgess Publishing Co.: Minneapolis, MN, USA, 1972.
33. Hiscox, J.; Israelstam, G.F. A method for the extraction of chlorophyll from leaf tissue without maceration. *Can. J. Bot.* **1979**, *57*, 1332–1334. [CrossRef]
34. Anonymous. *Recommended Practice for Chemical Analysis by Ion Chromatography*; Australian Standard AS 3741: Sidney, Australia, 1990.
35. Maxwell, K.; Johnson, G.N. Chlorophyll fluorescence—A practical guide. *J. Exp. Bot.* **2000**, *51*, 659–668. [CrossRef] [PubMed]

36. Bhatti, A.A.; Haq, S.; Bhat, R.A. Actinomycetes benefaction role in soil and plant health. *Microb. Pathog.* **2017**, *111*, 458–467. [CrossRef] [PubMed]
37. Charousová, I.; Javoreková, S.; Medo, J.; Schade, R. Characteristic of selected soil streptomycetes with antimicrobial potential against phytopathogenic microorganisms. *J. Microbiol. Biotechnol. Food Sci.* **2016**, *5*, 64–68. [CrossRef]
38. Charousová, I.; Medo, J.; Halenárová, E.; Javoreková, S. Antimicrobial and enzymatic activity of actinomycetes isolated from soils of coastal islands. *J. Adv. Pharm. Technol. Res.* **2017**, *8*, 46–51. [CrossRef]
39. AbdElgawad, H.; Abuelsoud, W.; Madany, M.M.Y.; Selim, S.; Zinta, G.; Mousa, A.S.; Hozzein, W.N. Actinomycetes Enrich Soil Rhizosphere and Improve Seed Quality as well as Productivity of Legumes by Boosting Nitrogen Availability and Metabolism. *Biomolecules* **2020**, *10*, 1675. [CrossRef] [PubMed]
40. Arunrat, N.; Sansupa, C.; Sereenonchai, S.; Hatano, R. Stability of soil bacteria in undisturbed soil and continuous maize cultivation in Northern Thailand. *Front. Microbiol.* **2023**, *14*, 1285445. [CrossRef] [PubMed]
41. Ivanov, D.A.; Bernards, M.A. Chlorophyll fluorescence imaging as a tool to monitor the progress of a root pathogen in a perennial plant. *Planta* **2015**, *243*, 263–279. [CrossRef] [PubMed]
42. Adhikari, K.; Owens, P.R.; Libohova, Z.; Miller, D.M.; Wills, S.; Nemecek, J.L. Assessing soil organic carbon stock of Wisconsin, USA and its fate under future land use and climate change. *Sci. Total Environ.* **2019**, *667*, 833–845. [CrossRef]
43. Hussain, M.I.; Reigosa, M.J. A chlorophyll fluorescence analysis of photosynthetic efficiency, quantum yield and photon energy dissipation in PSII antennae of *Lactuca sativa* L. leaves exposed to cinnamic acid. *Plant Physiol. Biochem.* **2011**, *49*, 1290–1298. [CrossRef] [PubMed]
44. Moustakas, M.; Bayçu, G.; Gevrek, N.; Moustaka, J.; Csatári, I.; Rognes, S.E. Spatiotemporal heterogeneity of photosystem II function during acclimation to zinc exposure and mineral nutrition changes in the hyperaccumulator *Noccaea caerulescens*. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 6613–6624. [CrossRef]
45. Oxborough, K.; Baker, N.R. Resolving chlorophyll a fluorescence images of photosynthetic efficiency into photochemical and non-photochemical components–calculation of qP and Fv0/Fm0 without measuring Fo0. *Photosynth. Res.* **1997**, *54*, 135–142. [CrossRef]
46. Del Pozo, A.; Pérez, P.P.; Gutiérrez, D.G.; Alonso, A.; Morcuende, R.; Martínez-Carrasco, R. Gas exchange acclimation to elevated CO_2 in upper-sunlit and lower-shaded canopy leaves in relation to nitrogen acquisition and partitioning in wheat grown in field chambers. *Environ. Exp. Bot.* **2007**, *59*, 371–380. [CrossRef]
47. White, P.J.; Broadley, M.R. Biofortification of crops with seven mineral elements often lacking in human diets—Iron, zinc, copper, calcium, magnesium, selenium and iodine. *New Phytol.* **2009**, *182*, 49–84. [CrossRef]
48. Kumssa, D.B.; Lovatt, J.A.; Graham, N.; Palmer, S.; Hayden, R.; Wilson, L.; Young, S.D.; Lark, R.M.; Penrose, B.; Ander, E.L.; et al. Magnesium biofortification of Italian ryegrass (*Lolium multiflorum* L.) via agronomy and breeding as a potential way to reduce grass tetany in grazing ruminants. *Plant Soil* **2019**, *457*, 25–41. [CrossRef]
49. Penrose, B.; Lovatt, J.A.; Palmer, S.; Thomson, R.; Broadley, M.R. Revisiting variation in leaf magnesium concentrations in forage grasses for improved animal health. *Plant Soil* **2020**, *457*, 43–55. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Humic Substances from Waste-Based Fertilizers for Improved Soil Fertility

Angela Maffia[1], Federica Marra[1], Francesco Canino[1], Santo Battaglia[1], Carmelo Mallamaci[1], Mariateresa Oliva [1] and Adele Muscolo [1]*

[1]Agriculture Department, Mediterranea University, 89124 Reggio Calabria, Italy;

*Correspondence: amuscolo@unirc.it

Agronomy

https://doi.org/10.3390/agronomy14112657

Received: 9 October 2024

Revised: 5 November 2024

Accepted: 7 November 2024

Published: 11 November 2024

agronomy

MDPI

Article

Humic Substances from Waste-Based Fertilizers for Improved Soil Fertility

Angela Maffia, Federica Marra, Francesco Canino, Santo Battaglia, Carmelo Mallamaci, Mariateresa Oliva and Adele Muscolo *

Agriculture Department, Mediterranea University, 89124 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); federica.marra@unirc.it (F.M.); francesco.canino@unirc.it (F.C.); santo.battaglia@unirc.it (S.B.); carmelo.mallamaci@unirc.it (C.M.); mariateresa.oliva@unirc.it (M.O.)
* Correspondence: amuscolo@unirc.it

Abstract: This research explores how different organic waste transformation methods influence the production of humic substances (HSs) and their impact on soil quality. Using olive and orange wastes as substrates, the study compares vermicomposting, composting, and anaerobic digestion processes to determine which method produces the most humic-substance-rich products. The characterization of HSs in each product included analyses of total organic carbon (TOC), humic and fulvic acid content, humification rate, humification degree, and E4/E6 ratio, with HSs extracted using potassium hydroxide (KOH) and analyzed via Diffuse Reflectance Infrared Fourier-Transform (DRIFT) spectroscopy to assess structural complexity. The results revealed that the chemical composition of the input materials significantly influenced the transformation dynamics, with orange by-products exhibiting a higher humification rate and degree. Vermicomposting emerged as the most efficient process, producing fertilizers with superior humic content, greater microbial biodiversity, and enhanced cation exchange capacity, thus markedly improving soil quality. Composting also contributed to the stabilization of organic matter, albeit less effectively than vermicomposting. Anaerobic digestion, by contrast, resulted in products with lower levels of HSs and reduced nutrient content. Aerobic processes, particularly vermicomposting, demonstrated the most rapid and effective transformation, producing structurally complex, stable humus-like substances with pronounced benefits for soil health. These findings underscore vermicomposting as the most sustainable and efficacious approach for generating HS-rich organic fertilizers, presenting a powerful alternative to synthetic fertilizers. Furthermore, this study highlights the potential of organic waste valorization to mitigate environmental pollution and foster circular economy practices in sustainable agriculture.

Keywords: organic wastes; vermicompost; anaerobic digestion; composting; humic substances; soil fertility

check for updates

Citation: Maffia, A.; Marra, F.; Canino, F.; Battaglia, S.; Mallamaci, C.; Oliva, M.; Muscolo, A. Humic Substances from Waste-Based Fertilizers for Improved Soil Fertility. *Agronomy* **2024**, *14*, 2657. https://doi.org/10.3390/agronomy14112657

Academic Editor: Maria Roulia

Received: 9 October 2024
Revised: 5 November 2024
Accepted: 7 November 2024
Published: 11 November 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The intensive use of synthetic chemicals, such as fertilizers and pesticides, in agriculture to promote crop growth and protect against pests, is accelerating the mineralization of organic matter, disrupting soil structure and decreasing soil biodiversity [1]. The soil is becoming less productive and more dependent on synthetic inputs to sustain crop yields [2]. Synthetic fertilizers often supply plants with readily available nutrients, but they can cause an imbalance in the soil's nutrient profile, resulting in nutrient leaching, and reducing soil fertility [3]. Runoff from fields treated with synthetic chemicals can lead to the contamination of water bodies. This pollution can harm aquatic ecosystems, reducing biodiversity, and affecting human health through contaminated drinking water [4]. The overuse of synthetic fertilizers and pesticides to respond the increasing demand of food, is decreasing the nutritional value of crops. Studies have shown that crops grown with excessive chemical inputs often have lower levels of essential vitamins and minerals [5,6].

Additionally, the presence of pesticide residues is posing health risks to consumers and is leading to the development of resistant pest populations. These resistant pests are harder to control, requiring even more potent chemicals, which exacerbates the cycle of chemical dependency and environmental harm [7].

The diminished nutritional value of crops and the presence of chemical residues can increase the risk of diseases in humans, leading to a range of health issues, including chronic diseases and developmental problems in children [8].

Efforts to address these issues include promoting sustainable agricultural practices, such as organic farming, integrated pest management, and the use of natural fertilizers and biopesticides. These practices aim to enhance soil health, improve crop quality, and reduce the negative environmental and health impacts associated with synthetic chemicals.

With the global population reaching 8 billion in 2022 and projected to grow to 9.7 billion by 2050, the demand for food and agricultural production continues to rise, leading to increased levels of organic waste generation [9]. This waste—comprising agricultural, market, and kitchen residues—decomposes quickly, yet improper management can result in environmental challenges, notably the emission of greenhouse gases that exacerbate climate change [10]. In Italy, as the second-largest European producer of oranges and olives, about 500,000 tons of orange waste and over 2000 tons of olive oil waste are produced annually [11]. Though non-toxic, these wastes contain high levels of polyphenols, low pH, and elevated salinity, which pose environmental risks [12]. A sustainable approach is to convert these wastes into eco-friendly fertilizers through composting, which not only reduces environmental harm but also improves soil quality [13], because sustainable waste management strategies are crucial for mitigating the impact on climate change and resource waste [14].

Aerobic composting is commonly employed to manage and repurpose organic matter in waste under thermophilic conditions, driven by the heat generated from biological processes [15,16]. Throughout the aerobic composting process, microorganisms transform raw organic materials into more stable substances, primarily humic substances (HSs) [17,18]. Vermicomposting is a highly sustainable process that transforms organic wastes into nutrient-rich compost enriched with humic substances through the activity of earthworms [19]. Anaerobic digestion of wastes involves the breakdown of organic material in the absence of oxygen, resulting in the production of biogas for energy and nutrient-dense digestate, which contains digestate that is rich in humic substances [20]. The humic substances, including humic acids and fulvic acids, are critical organic components that improve soil structure by forming aggregates, enhance nutrient availability, increase soil porosity, improve nutrient retention, water-holding capacity [21,22] and boost microbial activity, creating a fertile environment for plant growth [18]. Additionally, HSs act as natural pesticides, effectively suppressing various soil-borne phytopathogens and reducing the toxicity of chemical pesticides [23,24]. These benefits stem from the diverse functional groups present in HSs, such as carboxylic, phenolic, hydroxylic, and quinolyl groups [21].

The context of this research is focused on the urgent need for sustainable agricultural practices that enhance soil quality while reducing environmental impact. With rising concerns over soil degradation and excessive reliance on synthetic fertilizers, there has been a growing focus on organic waste transformation as a promising solution for generating humic-substance (HS)-enriched fertilizers. Current literature indicates that various organic waste transformation processes—such as composting, vermicomposting, and anaerobic digestion—yield differing levels of HSs [25–27], but there has been limited comparative analysis to determine which process optimally supports soil health.

This gap—understanding which waste transformation method best produces HS-rich fertilizers—shapes the primary research question: Which organic waste transformation method yields the most humic-substance-rich products and maximally enhances soil quality? This question is important to investigate because it addresses both the efficiency of these processes in producing beneficial soil amendments and the role of different substrates in optimizing outcomes.

Thus, this study aims to evaluate how different processes influence the conversion of organic wastes into humic substances (HSs). The research hypothesis posits that the transformation methods-vermicomposting, composting and anaerobic digestion- will yield different quantities and qualities of HSs, thereby affecting soil fertility. Specifically, olive and orange wastes were selected as matrices and subjected to these three processes to determine which method yields the highest HS-rich products. Additionally, the study examined the impact of chemically vermicompost, digestate and compost on soil quality, considering both the processing method and the type of waste used. Understanding the chemical characteristic of the HSs produced, including the amount and diversity of functional groups, can play a crucial role in shaping the agronomic effects of these waste-derived organic fertilizers when applied to soils. Answering this question has significant implications: it can help refine waste management practices, promote environmental sustainability, and foster circular economy models by converting agricultural waste into valuable soil amendments

2. Materials and Methods

2.1. Feeding Materials

Raw organic materials used for composting consisted of:

(1) 90% of olive pomace obtained from traditional three phases olive oil extraction process and 10% straw, as structuring material, named Compost 1 (C1); Olive pomace contained lignin (43%), hemicellulose (11.29%), cellulose (9.55%)
(2) 90% of orange wastes coming from the orange food industry, 10% straw as structuring material and manure, named Compost 2 (C2). Orange wastes contained lignin (19%), hemicellulose (7%) and cellulose (35%).

2.2. Composting Process Set Up

The composting processes were carried on in specialized electric composters designed to promote efficient decomposition. These composters feature separate chambers, which prevent the mixing of fresh and decomposing material, allowing independent temperature regulation in each chamber to optimize microbial activity.

The experiments were conducted in triplicate for each compost mixture under specific conditions: an initial mesophilic phase of 8 days at 29 °C, followed by a thermophilic phase of 20 days at 50 °C, and concluding with a prolonged mesophilic phase of 92 days at 27 °C [28]. Afterward, the compost entered a 30-day stabilization phase at a constant 20 °C to ensure maturity. During this phase, microbial activity diminished, and easily decomposable organic matter was depleted. Moisture levels were maintained at 50%, and oxygen concentrations stayed above 15%, with daily monitoring of temperature, moisture, and oxygen using a centrally placed probe. Water was added as necessary to maintain moisture, and daily mixing ensured sufficient oxygenation, promoting the breakdown of organic matter into stable humus. Once the composting process was complete, the compost was air-dried, finely ground to pass through a 2 mm sieve, and thoroughly mixed for uniformity. Both composts reached full maturity within six months [14].

2.3. Vermicomposting Process Set Up

The vermicomposting process was conducted using a 50-liter worm bin, specifically the Vevor 5-Tray Worm Composter (model WB25101). The bin was filled with a mixture consisting of:

(1) 45% of olive wastes, 45% organic food wastes, 45% straw (45:45:10) and earthworm 20%, named vermicompost 1 (V1),
(2) 45% orange wastes, 45% organic food wastes, 45% straw (45:45:10) and earthworm 20% named vermicompost 2 (V2),

with the bedding kept loose to promote airflow and maintained at a moisture level that was slightly damp but not excessively wet. Red wigglers (*Eisenia fetida*) were introduced to the bin at a density of 1000 worms (approximately one pound) per square foot of the bin's surface area. Over the course of four months, the organic material was broken down by the worms, resulting in mature vermicompost.

2.4. Digestate Process Set Up

The digestates were sourced from two biogas energy plants owned by the Fattoria della Piana cooperative in Candidoni, Calabria, Italy. Each plant operates with an installed capacity of 998 kW_e and a total volume of 3260 m^3. The plants were supplied with different feedstock compositions: the first plant received a mixture of 50% olive waste and 50% animal manure and maize silage, producing a digestate labeled as Digestate Olive (D1). The second plant was supplied with 50% orange waste, along with 50% animal manure and maize silage, yielding a digestate designated as Digestate Orange (D2).

The plant operators optimized process parameters, including temperature and retention time, to match the specific feedstock compositions. Both plants maintained a process temperature of 40 °C, with a daily loading volume of 120 m^3, a hydraulic retention time (HRT) of 60 days, and a minimum guaranteed retention time (MGRT) of 16 h at 40 °C. Following production, the digestates from both plants were analyzed to assess their chemical and biological properties, providing a basis for evaluating their suitability as humus-rich soil amendments.

2.5. Assessment of Chemical Characteristics of Composts, Vermicompost and Digestates

Chemical characterization of the initial wastes and composts was carried out according to the methodologies recommended by the ANPA manual [29]. Water soluble phenols (WSP), were detected by extracting soil in water, and determined by using the Folin–Ciocalteau reagent, following the Box Method [30]. Tannic acid was used as standard. Compost samples were extracted with bidistilled water (ratio compost/water 1:10) [31] for 24 h at 25 °C to detect ion concentration by using a chromatography system (Dionex ICS-1100). The NO_3–N was measured using a nitrate-ion selective electrode (USEPA, 2011), while NH_4-N was determined by a colorimetric method based on Berthelot's reaction [32].

2.6. Humic Substances Detection

Humic substances were extracted from the air-dried samples with 0.1 mol L^{-1} KOH (1:20 *w*/*v*) at room temperature for 16 h under a N_2 atmosphere and were separated from the suspended material by centrifugation at 7000× *g* for 20 min [33]. The humic extracts were chemically analyzed for total organic carbon, total extractable carbon, humic and fulvic acids humification rate, humification index, humification degree and E6/E4 ratio [34]. Diffuse Reflectance Infrared Fourier-Transform Spectroscopy (DRIFT) method was used to detect HS spectra. Spectra were recorded with a Nicolet Impact 400 Fourier-transform infrared spectrophotometer (Nicolet Instruments, Madison, WI, USA) and fitted with an apparatus for diffuse reflectance (Spectra-Tech, Stamford, CT, USA). Spectra were recorded with 200 scans collected at 4 cm^{-1} resolution, collected and manipulated by using the Omnic (Version 3.1) software supplied by Nicolet Instruments. For each analysis, 2 mg of dried sample was mixed with 148 mg of KBr (Fourier-transform infrared grade, Aldrich Chemical Co., Milwaukee, WI, USA) so that the mixture was homogeneous. The absorption bands of the HS were identified as described by others authors [34–37].

The CaOAc and $Ba(OH)^2$ methods [38] were used to determine the concentrations of carboxylic and total acidic functional groups, respectively [34]. The suspensions were filtered through 0.45 mm membrane filters as recommended by Perdue et al. [39]. Phenolic acidity was estimated as the difference between total and carboxyl acidity. Degree of humification (DH% = C(HA + HF) × 100/TEC) and humification rate (HR% = C(HA + HF) × 100/TOC) were calculated [40].

2.7. Soil Experiments

In this experiment a sandy-loam (11.85% clay, 23.21% silt, and 64.94% sand) soil was used [41]. The experiment was conducted from December to June with pots of 30 cm diameter each containing 9 kg of soil with a pH of 8.87 and 1.81% of organic matter and were amended with the different fertilizers whose dosage was chosen based on the carbon content and results obtained previously on various soils and different crops [12,42]. The experimental design employed a randomized block design with three replications for each treatment. This design allows the control of variability within the experimental units, enhancing the precision of treatment effect estimates.

The fertilizer amount used for each treatment and pot was:

Composts: 15 g per pot
Vermicompost: 12.5 g per pot
Digestate: 15 g per pot
Unfertilized pots have been used for comparison.

The application of fertilizers was performed uniformly to ensure consistent treatment across all experimental units. For each pot, the designated amount of fertilizer was carefully weighed and then incorporated into the soil to a dept of 7 cm, simulating typical field incorporation practices. After application, the soil was gently mixed to promote even distribution of each fertilizer within the topsoil layer, ensuring optimal contact between soil particles and the amendment.

Six months after the experiment began, in June, soil samples were collected, air-dried, and sieved through a 2 mm mesh for chemical and enzyme activity analysis. The experiments were performed in triplicates in greenhouse situated in Mediterranea University of Reggio Calabria at 25 °C day/19 °C night [43]. During the experiment, the pots were watered regularly to ensure that water content was maintained at 70% of field capacity, (Figure 1). At the end of the experiments (180 days after treatments) the differently treated soils (three replicates), were air-dried and sieved (<2 mm) prior to the chemical analysis (fully described in the section soil and pad analysis). Soil samples for the biochemical determination (microbial biomass and enzyme activities) were stored in the refrigerator at 4 °C for up to 24 h until processing. Each chemical and biological properties have been analyzed in 3 replicates. In this experiment, three pots filled with soil and treated with different fertilization types are sufficient as replicates due to the controlled environment provided by the climate chamber. In a climate chamber, variables such as temperature, humidity, and light are precisely regulated, minimizing external fluctuations and ensuring consistent conditions across all treatments. This control reduces the variability typically introduced by environmental factors, allowing for reliable comparisons between the differently fertilized pots even with a small number of replicates.

Moreover, analyzing each soil sample in triplicate further supports the experiment's validity. Triplicate analyses provide a robust measure of the soil properties within each pot, reducing the influence of random errors and increasing the reliability of the results. Together, the controlled conditions of the climate chamber and the triplicate analysis of each soil sample strengthen the experimental design, ensuring that the findings are both accurate and reproducible even with a smaller number of initial replicates.

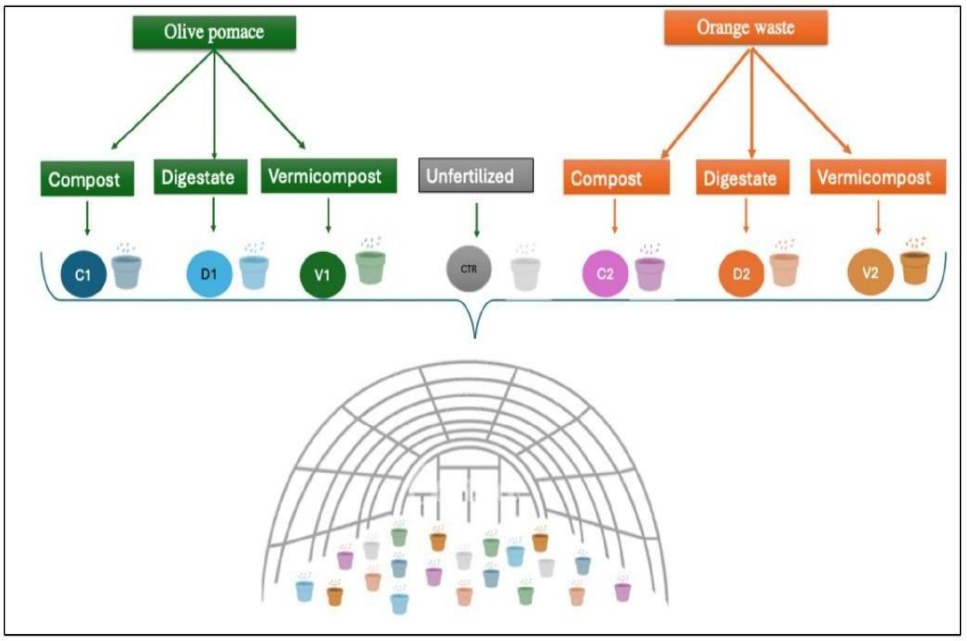


Figure 1. Experimental design in greenhouse. CTR unfertilized soil; C1 (90% wastes from olive oil + 10% straw), C2 (90% orange waste and 10% of straw), D1 (Digestate coming from olive waste 50%, and animal manure and maize silage 50%), D2 (Digestate coming from orange waste 50%, animal manure and maize silage 50%), V1 (vermicompost olive wastes: organic food waste: straw (45:45:10) earthworm 20%), V2 (vermicompost orange wastes: organic food wastes: straw (45:45:10) earthworm 20%.

2.8. Soil Analyses

Six months post-treatment, soil samples were analyzed for their physical and biological properties. Various soil parameters were measured, including pH, electrical conductivity (EC) [44], organic carbon (OC) content [45], total nitrogen (TN) content [46], and the carbon content in humic and fulvic acids [47]. Additional analyses included water-soluble phenols (WSP) [30] and cation exchange capacity (CEC) [48].

Enzyme activity, specifically fluorescein diacetate (FDA) hydrolysis, was measured [49]. Microbial biomass carbon (MBC) was assessed using the chloroform-fumigation extraction procedure [50]. Fumigated and unfumigated soil extracts were analyzed for soluble organic carbon [45], with MBC calculated from the difference in organic C between the fumigated and unfumigated samples, applying an extraction efficiency coefficient of 0.38 [50]. Dehydrogenase (DH) activity was determined in soil sample differently amended [51].

Anions, including NO_{3^-}, and cations, including NH_{4^+}, were detected using ion chromatography [43], using a DIONEX ICS-1100 chromatography system (Thermo Fisher Scientific, Waltham, MA, USA). NH_4-N was calculated as the mass of the element divided by the molecular mass of the compound. Cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were extracted and analyzed using ion chromatography after incinerating 1 g of dried sample at 550 °C for 6 h. The resulting ash was acidified with 1M HCl for 30 min at 100 °C, filtered, and analyzed with 20 mM methane-sulfonic acid as the eluent. Iron (Fe) levels were measured via atomic absorption spectrophotometry using a PerkinElmer 2380 device (Waltham, MA, USA). Calibration curves were constructed with mixed standard solutions (1, 5, 25, and 50 ppm) for each anion, ensuring accurate correlation between peak area and concentration. Cation concentrations were derived from their respective standard curves [52]. All chemicals and reagents were sourced from Panreac Quìmica SLU, Barcelona, Spain.

2.9. Statistical Analysis

Data are expressed as means of three analyses for each treatment. To analyze the effect of different fertilizers to various parameters measured, a Two-way analysis of variance ANOVA with Tukey's Honestly Significant Difference (HSD) test was conducted. This statistical approach allows for comparison across treatments while accounting for potential interaction between the types of organic waste and the transformation processes. The analyses were carried out using XLStat with significance differences at $p \leq 0.05$. To explore. Relationships among different fertilizers on soil parameter datasets we analyzed using Principal Component Analysis (PCA) with XLStat.

3. Results

3.1. Characteristics of Composts, Vermicompost and Digestates

The results on the chemical characteristics of compost, vermicompost, and digestate revealed significant variations, even within the same product category, largely due to differences in the waste materials used. Products derived from orange residues showed higher concentrations of cations, anions, carbon, nitrogen, and a higher C/N ratio (Table 1) compared to those derived from olive waste. In contrast, compost, vermicompost, and digestate produced from olive waste had the highest levels of water-soluble phenols (Table 1). These chemical differences between products made from the same type of waste but processed differently underscore the critical role of the transformation process—its specific setup and conditions—in shaping the final chemical composition, even when the waste material is the same.

Table 1. Chemical characteristic of C1, C2, D1, D2, V1, V2. The data are the mean of 3 replicates ± standard deviation ($n = 18$). Different letters in the same row indicate, significant differences among the treatments (Tukey's test, $p \leq 0.05$).

Chemical Characteristics	C1	C2	V1	V2	D1	D2
pH (H_2O)	$6.3^{c} \pm 0.05$	$7.6^{b} \pm 0.5$	$7.49^{b} \pm 0.03$	$7.61^{b} \pm 0.02$	$8.5^{a} \pm 0.2$	$8.3^{a} \pm 0.8$
BD (kg × m^{-3})	$598^{c} \pm 9.0$	$558^{c} \pm 12$	$554^{b} \pm 9$	$577^{b} \pm 8$	$788^{a} \pm 9.2$	$768^{a} \pm 11$
EC (mS × cm^{-1})	$1.3^{b} \pm 0.25$	$1.8^{b} \pm 0.2$	$3.18^{a} \pm 0.2$	$2.5a^{b} \pm 0.4$	$1.3^{b} \pm 0.2$	$1.5^{b} \pm 0.4$
WC (%)	$47^{b} \pm 3.2$	$44^{b} \pm 3$	$49.6^{b} \pm 2$	$42.3^{b} \pm 1$	$64^{a} \pm 7$	$69^{a} \pm 6$
TC (%)	$44^{b} \pm 2.40$	$49^{b} \pm 2.4$	$52.8^{a} \pm 1.2$	$59.5^{a} \pm 2$	$45^{b} \pm 1.4$	$47^{b} \pm 1.4$
TN (%)	$2.5^{ab} \pm 0.22$	$2.7^{ab} \pm 0.8$	$2.13^{b} \pm 0.03$	$2.66^{ab} \pm 0.02$	$3.7^{a} \pm 0.2$	$2.2^{a} \pm 0.9$
C/N	$17.6^{ab} \pm 1.6$	$18.1^{ab} \pm 1.6$	$19.19^{ab} \pm 0.6$	$22.03^{a} \pm 0.8$	$12.1^{b} \pm 0.5$	$21.4^{a} \pm 0.9$
Na^{+} (mg g^{-1} dw)	$1.1^{c} \pm 0.06$	$0.9^{c} \pm 0.02$	$4.69^{a} \pm 0.09$	$2.39^{b} \pm 0.07$	$0.9^{c} \pm 0.08$	$0.8^{c} \pm 0.1$
NH_4^{+} (mg g^{-1} dw)	$0.7^{b} \pm 0.02$	$0.6^{b} \pm 0.01$	$0.5^{a} \pm 0.04$	$0.33^{b} \pm 0.04$	$1.5^{a} \pm 0.02$	$1.53^{a} \pm 0.01$
K^{+} (mg g^{-1} dw)	$17^{a} \pm 1.50$	$18^{a} \pm 1.3$	$7.57^{b} \pm 0.2$	$9.65^{b} \pm 0.3$	$0.5^{d} \pm 0.02$	$3.6^{c} \pm 0.5$
Mg^{2+} (mg g^{-1} dw)	$1.1a^{b} \pm 0.1$	$1.8^{a} \pm 0.2$	$0.37^{b} \pm 0.02$	$1.22^{ab} \pm 0.04$	$0.53^{b} \pm 0.08$	$0.85^{b} \pm 0.06$
Ca^{2+} (mg g^{-1} dw)	$2.4^{a} \pm 0.3$	$2.9^{a} \pm 0.2$	$0.21^{b} \pm 0.03$	$2.3^{a} \pm 0.06$	$1.7^{a} \pm 0.2$	$1.8^{a} \pm 0.1$
Cl^{-} (mg g^{-1} dw)	nd	nd	$11.12^{a} \pm 0.9$	$9.23^{a} \pm 1.1$	nd	$0.48^{b} \pm 0.05$
NO_2^{-} (mg g^{-1} dw)	nd	nd	nd	0.33 ± 1.1	nd	nd
NO_3^{-} (mg g^{-1} dw)	$0.42^{b} \pm 0.002$	$0.51^{a} \pm 0.01$	nd	$0.87^{a} \pm 1.1$	$0.21^{b} \pm 0.03$	$0.42^{a} \pm 0.02$
PO_4^{3-} (mg g^{-1} dw)	$0.43^{b} \pm 0.03$	$0.90^{ab} \pm 0.03$	$1.25^{a} \pm 0.01$	$1.44^{a} \pm 0.02$	$0.47^{b} \pm 0.06$	$0.63^{b} \pm 0.04$
SO_4^{2-} (mg g^{-1} dw)	$0.27^{b} \pm 0.02$	$0.87^{ab} \pm 0.02$	$0.93a^{b} \pm 0.02$	$1.33^{a} \pm 0.02$	$0.11^{b} \pm 0.01$	$0.44^{ab} \pm 0.02$
WSP (mg TAE g^{-1} dw)	$2.42^{ab} \pm 0.06$	$1.0^{b} \pm 0.6$	$2.17^{b} \pm 0.03$	$1.77^{b} \pm 0.05$	$5.4^{a} \pm 1$	$2.1^{b} \pm 0.5$
ON/TN	$93^{a} \pm 5$	$99^{a} \pm 3$	$95.3^{a} \pm 2$	$96.1^{a} \pm 3$	$92^{a} \pm 8$	$93^{a} \pm 5$
NH_4^{+}-N/NO_3^{-}-N	$1.66^{c} \pm 0.13$	$1.17^{c} \pm 0.13$	nd	$0.37^{c} \pm 0.05$	$7.14^{a} \pm 0.07$	$3.64^{b} \pm 0.05$

pH (H_2O), Bulk Density (BD), Electrical conductibility (EC), Water content (WC), Total Carbon (TC); Total Nitrogen (TN), Carbon/Nitrogen ratio (C/N), Water Soluble Phenols (WSP), Organic nitrogen/total nitrogen (ON/TN), Ammonium-Nitrate-Nitrogen ratio (NH_4^{+}-N/NO_3^{-}).

A Pearson correlation matrix (Table 2) of the chemical properties of compost, vermicompost, and digestate showed that pH shows a strong negative correlation with K^{+} and NH4+-N/NO_3^{-}-N indicating that as pH increases, the concentration of potassium and the ammonium/nitrate ratio decreases. This suggests that alkaline conditions may hinder

the availability of potassium and shift nitrogen towards nitrate forms. However, EC was positively correlated with Na, Cl, and PO_4^{2-}, and WC correlated with bulk density while showing an inverse correlation with NH_4^+. TOC was linked to carbon and anions, while TN was inversely correlated with the C/N ratio. Additionally, ON/TN correlated with ammonium, and the NH_4^+-N/NO_3^--N ratio was positively correlated with potassium (Table 2). The humic characteristics of the six fertilizers further revealed that those produced from olive waste contained lower levels of carbon, humic acids, and fulvic acids compared to fertilizers derived from orange residues, humic acids were more abundant in digestate than compost and vermicompost, conversely vermicompost had the greatest percentage of fulvic acids. Fertilizers from orange waste exhibited the highest humification rate and degree, while those made from olive waste had the highest humification index (HI) and E4/E6 ratio (Table 3). A Pearson correlation matrix of the humic characteristics across the different fertilizers (Table 4) showed a positive correlation among all parameters, except for HI and E4/E6. These two were negatively correlated with the other chemical parameters but positively correlated with each other (Table 4).

3.2. Effect of Composts, Vermicompost and Digestates on Soil

Soil analysis results also demonstrated significant differences across the treatments (Table 5). There was no change in soil texture and pH compared to CTR0 and CTR, but an increase in EC values and water-soluble phenols (WSP), especially with olive compost. Water content, TOC, TN, and SOM increased in compost-treated soils compared to both controls, and markers of active soil life such as MBC, FDA, and DHA were highest in soils treated with compost (Figure 2). The HC/FC ratio and CEC were also elevated in compost-treated soils (Table 5). Similar trends were observed with vermicompost treatments (Table 5), except that FDA and DHA increased only with vermicompost from orange waste (Figure 2). HC and CEC increased with both vermicomposts, but the HC/FC value increased only with orange vermicompost (Table 5).

A Pearson correlation matrix of chemical soil properties at starting of the experiments, not amended and fertilized evidenced numerous correlations. pH was always inversely correlated with the other soil parameters, EC only with WSP and TN. WC as expected was correlated with TOC, SOM, HC/FC and CEC. TOC was positively correlated with SOM, WC, and CEC, WSP showed positive correlation with MBC, TN and CEC. DHA and FDA correlated positively each other (Table 6).

PCA analysis of different fertilizers obtained with the different matrix and processes, evidenced that orange compost correlated with nutrients, both vermicompost correlated with TC, SOM and anions, while digestates correlated with WSP, WC and BD (Figure 3). The chemical characteristics of humic substances contained into the different fertilizers showed as V1 and V2 contained better quality humus as shown by TOC, TEC, CHA + CFA (Figure 4). The PCA related to potted soil differently amended, starting soil and control soil, evidenced as V1 and V2 better affected soil properties in terms of organic matter, TN, CEC HC/FC MBC and WC (Figure 5).

Table 2. Correlation matrix (Pearson (n)) of chemical characteristic of: C1, C2, D1, D2, V1, V2. Green color and its shades, in the correlation matrix, indicate a positive correlation, signifying that the variables move in the same direction. The red color and its gradients represent an inverse correlation, suggesting variables move in the opposite direction.

Variables	pH	BD	EC	WC	TC	TN	C/N	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	WSP	ON/TN	NH_4^+-N/NO_3^--N
pH	1	0.686	−0.062	0.679	0.048	0.403	−0.188	−0.153	−0.386	−0.798	−0.291	−0.197	−0.074	−0.015	0.222	0.007	−0.092	0.421	−0.146	−0.776
BD	0.686	1	−0.622	0.939	−0.521	0.472	−0.41	−0.534	−0.891	−0.775	−0.424	−0.05	−0.527	−0.288	−0.055	−0.63	−0.705	0.709	−0.74	−0.382
EC	−0.062	−0.622	1	−0.455	0.785	−0.477	0.465	0.942	0.588	0.002	−0.239	−0.613	0.955	0.368	0.069	0.897	0.785	−0.389	0.455	−0.469
WC	0.679	0.939	−0.455	1	−0.528	0.205	−0.278	−0.345	−0.909	−0.791	−0.543	−0.278	−0.418	−0.456	−0.32	−0.57	−0.669	0.574	−0.707	−0.497
TC	0.048	−0.521	0.785	−0.528	1	−0.269	0.622	0.585	0.568	0.024	0.111	−0.136	0.837	0.841	0.629	0.968	0.945	−0.439	0.525	−0.411
TN	0.403	0.472	−0.477	0.205	−0.269	1	−0.82	−0.445	−0.103	−0.326	−0.049	0.264	−0.397	0.01	0.435	−0.368	−0.415	0.776	−0.258	0.029
C/N	−0.188	−0.41	0.465	−0.278	0.622	−0.82	1	0.286	0.155	0.236	0.271	0.022	0.468	0.497	0.132	0.604	0.692	−0.813	0.379	−0.164
Na^+	−0.153	−0.534	0.942	−0.345	0.585	−0.445	0.286	1	0.444	−0.081	−0.483	−0.79	0.922	0.181	−0.138	0.714	0.551	−0.187	0.199	−0.434
NH_4^+	−0.386	−0.891	0.588	−0.909	0.568	−0.103	0.155	0.444	1	0.639	0.476	0.127	0.472	0.337	0.29	0.667	0.724	−0.512	0.854	0.261
K^+	−0.798	−0.775	0.002	−0.791	0.024	−0.326	0.236	−0.081	0.639	1	0.764	0.549	−0.112	0.018	−0.061	0.089	0.288	−0.686	0.624	0.836
Mg^{2+}	−0.291	−0.424	−0.239	−0.543	0.111	−0.049	0.271	−0.483	0.476	0.764	1	0.879	−0.34	0.229	0.34	0.081	0.333	−0.63	0.703	0.616
Ca^{2+}	−0.197	−0.05	−0.613	−0.278	−0.136	0.264	0.022	−0.79	0.127	0.549	0.879	1	−0.611	0.219	0.437	−0.258	−0.019	−0.258	0.307	0.662
Cl^-	−0.074	−0.527	0.955	−0.418	0.837	−0.397	0.468	0.922	0.472	−0.112	−0.34	−0.611	1	0.539	0.218	0.886	0.754	−0.255	0.266	−0.504
NO_2^-	−0.015	−0.288	0.368	−0.456	0.841	0.01	0.497	0.181	0.337	0.018	0.229	0.219	0.539	1	0.881	0.685	0.712	−0.229	0.257	−0.182
NO_3^-	0.222	−0.055	0.069	−0.32	0.629	0.435	0.132	−0.138	0.29	−0.061	0.34	0.437	0.218	0.881	1	0.45	0.493	0.031	0.245	−0.122
PO_4^{3-}	0.007	−0.63	0.897	−0.57	0.968	−0.368	0.604	0.714	0.667	0.089	0.081	−0.258	0.886	0.685	0.45	1	0.964	−0.509	0.622	−0.41
SO_4^{2-}	−0.092	−0.705	0.785	−0.669	0.945	−0.415	0.692	0.551	0.724	0.288	0.333	−0.019	0.754	0.712	0.493	0.964	1	−0.677	0.752	−0.226
WSP	0.421	0.709	−0.389	0.574	−0.439	0.776	−0.813	−0.187	−0.512	−0.686	−0.63	−0.258	−0.255	−0.229	0.031	−0.509	−0.677	1	−0.745	−0.243
ON/TN	−0.146	−0.74	0.455	−0.707	0.525	−0.258	0.379	0.199	0.854	0.624	0.703	0.307	0.266	0.257	0.245	0.622	0.752	−0.745	1	0.169
NH_4^+-N/NO_3^--N	−0.776	−0.382	−0.469	−0.497	−0.411	0.029	−0.164	−0.434	0.261	0.836	0.616	0.662	−0.504	−0.182	−0.122	−0.41	−0.226	−0.243	0.169	1

Table 3. Humic characteristics of the six fertilizers obtained with the different processes and biomass of C1, C2, D1, D2, V1, V2. The data are the mean of 3 replicates ± standard deviation (n = 18). Different letters in the same row indicate, significant differences among the treatments (Tukey's test, $p \leq 0.05$).

	C1	C2	V1	V2	D1	D2
TOC (%)	$44^{b} \pm 1.4$	$49^{ab} \pm 1.9$	$53^{a} \pm 2.4$	$59^{ba} \pm 1.9$	$45^{b} \pm 1.5$	$47^{b} \pm 1.2$
TEC (%)	$17^{b} \pm 1$	$22^{a} \pm 1.4$	$24^{a} \pm 1.1$	$28^{a} \pm 1.5$	$18^{b} \pm 1$	$20^{ab} \pm 1.5$
HA + FA	$11^{c} \pm 1$	$18^{b} \pm 1.6$	$19^{b} \pm 1.5^{b}$	$25^{a} \pm 1.4$	$11^{c} \pm 1$	$12^{c} \pm 1$
HA	$6^{c} \pm 0.5$	$10^{a} \pm 1.4$	$8^{b} \pm 0.9$	$9^{ab} \pm 0.9$	$9^{ab} \pm 1$	$10^{a} \pm 1$
FA	$5^{d} \pm 0.3$	$8^{c} \pm 0.4$	$11^{b} \pm 1.0$	$16^{a} \pm 1.1$	$2^{e} \pm 0.2$	$2^{e} \pm 0.1$
HR (%)	$25^{b} \pm 1.8$	$36^{a} \pm 1.5$	$35^{a} \pm 1$	$39^{a} \pm 1.5$	$24^{b} \pm 1.4$	$26^{b} \pm 1.5$
HD (%)	$64^{b} \pm 1.9$	$80^{a} \pm 2.9$	$81^{a} \pm 2.5$	$87^{a} \pm 2$	$61^{b} \pm 1.9$	$63^{b} \pm 2$
HI (%)	$0.57^{a} \pm 0.01$	$0.25^{b} \pm 0.04$	$0.22^{b} \pm 0.04$	$0.15^{b} \pm 0.03$	$0.63^{a} \pm 0.06$	$0.58^{a} \pm 0.05$
E4/E6	$7.3^{a} \pm 0.9^{a}$	$4.5^{b} \pm 0.6^{b}$	$6.1^{a} \pm 0.8$	$4.2^{b} \pm 0.7$	$9.3^{a} \pm 1.0$	$7.1^{a} \pm 0.9$
TA (mol kg^{-1})	$9.5^{ab} \pm 0.8$	$8.9^{b} \pm 0.4$	$10.1^{a} \pm 0.5$	$11.4^{a} \pm 0.6$	$6.5^{c} \pm 0.8$	$6.9^{c} \pm 0.8$
OH (mol kg^{-1})	$5.4^{a} \pm 0.1$	$2.1^{b} \pm 0.5$	$4.9^{a} \pm 0.4$	$4.2^{a} \pm 0.4$	$5.1^{a} \pm 0.3$	$4.9^{a} \pm 0.2$
COOH (mol kg^{-1})	$4.1^{b} \pm 0.5$	$6.8^{a} \pm 0.4$	$5.2^{ab} \pm 0.6$	$7.4^{a} \pm 0.3$	$1.4^{c} \pm 0.5$	$2^{c} \pm 0.7$

Total Organic Carbon (TOC), Total extractable Carbon (TEC); Humic acid carbon (HA); fulvic acid carbon (FA); humic acid carbon (HA) + fulvic acid carbon (FA), humification rate (HR), humification degree (HD), humification index (HI), E4/E6 (ratio of the absorbances at 465 and 665 nm), total acidity (TA), phenolic OH groups (OH), carboxylic groups (COOH).

Table 4. Correlation matrix (Pearson (n − 1)) of humic characteristics of C1, C2, D1, D2, V1, V2 obtained with the different processes and biomass. Green color and its shades, in the correlation matrix, indicate a positive correlation, signifying that the variables move in the same direction. On the other hand, the red color and its gradients represent an inverse correlation, suggesting that the variables move in the opposite direction.

Variables	TOC	TEC	HA + FA	HA	FA	HR	HD	HI	E4/E6	TA	OH	COOH
TOC	1	0.992	0.970	0.235	0.933	0.889	0.900	−0.880	−0.755	0.759	−0.261	0.738
TEC	0.992	1	0.978	0.325	0.916	0.922	0.922	−0.907	−0.801	0.725	−0.360	0.760
HA + FA	0.970	0.978	1	0.235	0.964	0.965	0.971	−0.954	−0.857	0.807	−0.450	0.867
HA	0.235	0.325	0.235	1	−0.032	0.277	0.181	−0.185	−0.248	−0.354	−0.575	0.014
FA	0.933	0.916	0.964	−0.032	1	0.917	0.949	−0.931	−0.813	0.927	−0.306	0.888
HR	0.889	0.922	0.965	0.277	0.917	1	0.994	−0.992	−0.918	0.776	−0.617	0.920
HD	0.900	0.922	0.971	0.181	0.949	0.994	1	−0.997	−0.896	0.828	−0.547	0.926
HI	−0.880	−0.907	−0.954	−0.185	−0.931	−0.992	−0.997	1	0.893	−0.813	0.558	−0.918
E4/E6	−0.755	−0.801	−0.857	−0.248	−0.813	−0.918	−0.896	0.893	1	−0.759	0.687	−0.940
TA	0.759	0.725	0.807	−0.354	0.927	0.776	0.828	−0.813	−0.759	1	−0.173	0.875
OH	−0.261	−0.360	−0.450	−0.575	−0.306	−0.617	−0.547	0.558	0.687	−0.173	1	−0.628
COOH	0.738	0.760	0.867	0.014	0.888	0.920	0.926	−0.918	−0.940	0.875	−0.628	1

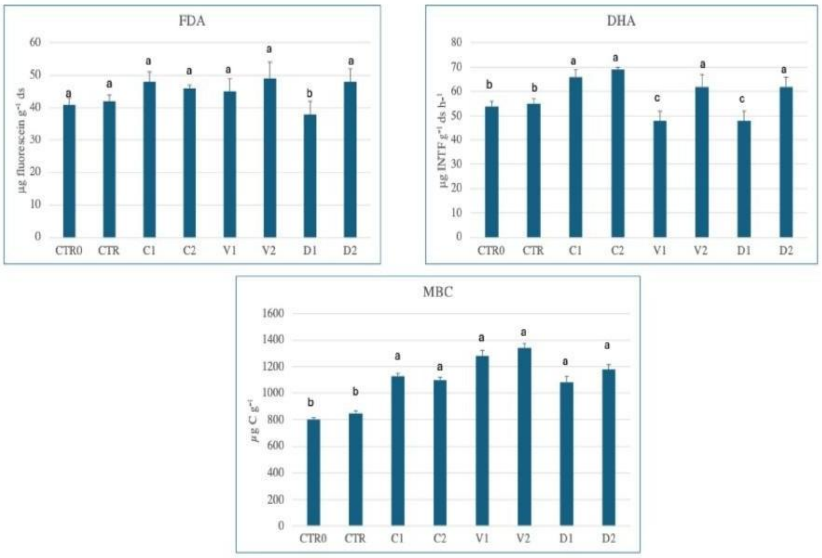


Figure 2. Fluorescein Hydrolase activity (FDA, μg fluorescein g^{-1} ds), Dehydrogenase activity (DHA, μg INTF g^{-1} ds h^{-1}), Microbial Biomass Carbon (MB μg C g^{-1} fs), of potted alkaline sandy-loam soils CTR0, and six months after the addition of C1, C2, D1, D2, V1, V2. Not amended soil was used as control (CTR). The data are the mean of three replicates ± standard deviation. Different letters significant differences among the treatments (Tukey's test, $p \leq 0.05$).

Table 5. Physical and chemical properties of potted alkaline sandy-loam soils CTR0, and six months after the addition of: C1, C2, D1, D2, V1, V2. Not amended soil was used as control (CTR). The data are the mean of three replicates ± standard deviation (n = 24). Different letters in the same row indicate, significant differences among the treatments (Tukey's test, $p \leq 0.05$).

	CTR0	CTR	C1	C2	V1	V2	D1	D2
pH (H_2O)	8.3 ^{a} ± 0.55	8.3 ^{a} ± 0.52	7.5 ^{a} ± 0.80	8.0 ^{a} ± 0.80	7.6 ^{ab} ± 0.40	7.3 ^{b} ± 0.40	7.1 ^{b} ± 0.40	7.4 ^{b} ± 0.40
EC (dS/m)	320 ^{c} ± 10	340 ^{c} ± 12	444 ^{a} ± 9	410 ^{a} ± 10	380 ^{b} ± 12	367 ^{b} ± 12	419 ^{a} ± 12	437 ^{a} ± 12
WC (%)	21.0 ^{b} ± 2.6	22 ^{b} ± 2.1	27 ^{a} ± 1.7	29.4 ^{a} ± 0.79	27.2 ^{a} ± 1.70	29.2 ^{a} ± 1.70	24 ^{a} ± 1.70	21 ^{a} ± 1.70
WSP (μg TAE g^{-1} ds)	18 ^{b} ± 2.0	14 ^{b} ± 2.8	46 ^{a} ± 1.7	40 ^{a} ± 1.60	39 ^{a} ± 3.3	34 ^{a} ± 3.1	40 ^{a} ± 3.26	39 ^{a} ± 3.26
TOC (%)	1.0 ^{bc} ± 0.16	0.9 ^{c} ± 0.16	1.7 ^{b} ± 0.15	2.1 ^{a} ± 0.15	2.1 ^{a} ± 0.25	2.5 ^{a} ± 0.25	1.3 ^{b} ± 0.25	1.5 ^{b} ± 0.25
TN (%)	0.13 ^{c} ± 0.01	0.14 ^{c} ± 0.01	0.30 ^{a} ± 0.02	0.33 ^{a} ± 0.02	0.22 ^{b} ± 0.04	0.23 ^{b} ± 0.04	0.21 ^{b} ± 0.04	0.25 ^{b} ± 0.04
C/N	7.6 ^{ab} ± 0.35	6.4 ^{b} ± 0.4	5.7 ^{c} ± 1	6.3 ^{b} ± 0.3	9.5 ^{a} ± 0.4	10.9 ^{a} ± 0.5	6.2 ^{b} ± 0.5	6.1 ^{b} ± 0.3
SOM (%)	1.72 ^{c} ± 0.3	1.55 ^{c} ± 0.27	2.92 ^{b} ± 0.25	3.62 ^{ab} ± 0.3	3.6 ^{ab} ± 0.13	4.3 ^{a} ± 0.4	2.24 ^{cb} ± 0.13	2.58 ^{cb} ± 0.4
HC (%)	0.60 ^{a} ± 0.06	0.61 ^{a} ± 0.05	0.43 ^{b} ± 0.02	0.44 ^{b} ± 0.02	0.70 ^{a} ± 0.01	0.75 ^{a} ± 0.01	0.66 ^{a} ± 0.01	0.65 ^{a} ± 0.01
FC (%)	0.40 ^{a} ± 0.06	0.45 ^{a} ± 0.08	0.26 ^{b} ± 0.05	0.22 ^{b} ± 0.05	0.38 ^{a} ± 0.03	0.35 ^{a} ± 0.05	0.62 ^{a} ± 0.03	0.60 ^{a} ± 0.03
HC/FC	1.5 ^{b} ± 0.12	1.33 ^{bc} ± 0.12	1.65 ^{b} ± 0.10	2 ^{a} ± 0.10	1.84 ^{b} ± 0.04	2.14 ^{a} ± 0.04	1.06 ^{c} ± 0.04	1.08 ^{c} ± 0.04
CEC ($cmol(^{+})\ kg^{-1}$)	18.9 ^{b} ± 1.6	18.7 ^{b} ± 1.4	22 ^{a} ± 1.6	24 ^{a} ± 1.5	23 ^{a} ± 1.23	25 ^{a} ± 1.3	22 ^{a} ± 1.23	23 ^{a} ± 1.3

pH (H_2O), Electrical conductibility (EC), Water content (WC), Water soluble phenols (WSP), Total Organic Carbon (TOC), Total Nitrogen (TN), Soil Organic Matter (SOM); Carbon/Nitrogen ratio (C/N); Humic content (HC), Fulvic Content (FC), Humic content/Fulvic Content ratio (HC/FC), Cation Exchange capacity (CEC).

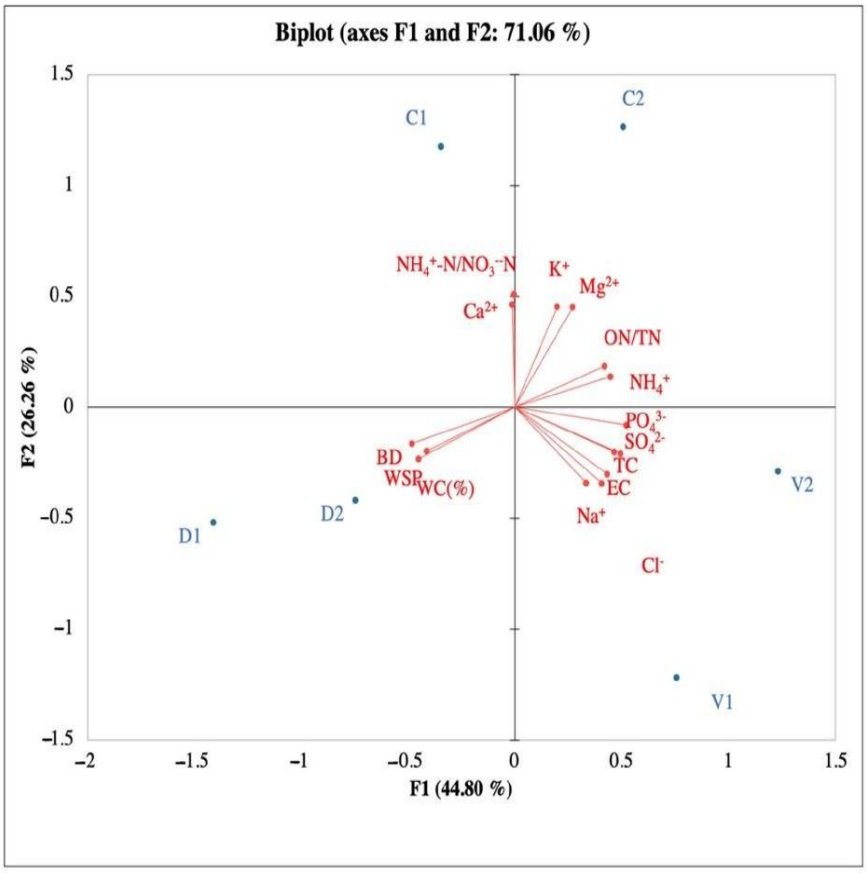


Figure 3. PCA of chemical characteristic of: C1, C2, D1, D2, V1 and V2.

Table 6. Correlation matrix (Pearson (n)) of chemical properties of potted alkaline sandy-loam soils CTR0, not amended soil was used as control (CTR) and six months after the addition of: C1, C2, D1, D2, V1 and V2. Green color and its shades, in the correlation matrix, indicate a positive correlation, signifying that the variables move in the same direction. On the other hand, the red color and its gradients represent an inverse correlation, suggesting that the variables move in the opposite direction.

Variables	pH	EC	WC	WSP	TOC	TN	C/N	SOM	FDA	DHA	MBC	HC	FC	HC/FC	CEC
pH	1	−0.675	−0.316	**−0.767**	−0.465	−0.41	−0.146	−0.464	−0.225	0.067	**−0.764**	−0.364	−0.358	0.096	−0.639
EC	−0.675	1	0.275	**0.893**	0.296	**0.804**	−0.477	0.297	0.367	0.392	0.515	−0.348	0.077	−0.185	0.487
WC	−0.316	0.275	1	0.568	**0.888**	0.689	0.414	**0.889**	0.487	0.396	0.675	−0.155	−0.687	**0.836**	**0.845**
WSP	**−0.767**	**0.893**	0.568	1	0.604	**0.844**	−0.109	0.603	0.414	0.289	**0.74**	−0.196	−0.113	0.146	**0.738**
TOC	−0.465	0.296	**0.888**	0.604	1	0.652	0.599	1	0.693	0.38	**0.883**	0.139	−0.495	**0.795**	**0.95**
TN	−0.41	**0.804**	0.689	**0.844**	0.652	1	−0.212	0.654	0.612	0.701	0.593	−0.517	−0.46	0.38	**0.737**
C/N	−0.146	−0.477	0.414	−0.109	0.599	−0.212	1	0.597	0.261	−0.232	0.494	0.692	−0.193	0.644	0.432
SOM	−0.464	0.297	**0.889**	0.603	1	0.654	0.597	1	0.692	0.383	**0.882**	0.138	−0.495	**0.795**	**0.951**
FDA	−0.225	0.367	0.487	0.414	0.693	0.612	0.261	0.692	1	**0.724**	0.645	−0.104	−0.47	0.544	0.593
DHA	0.067	0.392	0.396	0.289	0.38	0.701	−0.232	0.383	**0.724**	1	0.175	−0.611	−0.581	0.403	0.375
MBC	**−0.764**	0.515	0.675	**0.74**	**0.883**	0.593	0.494	**0.882**	0.645	0.175	1	0.343	−0.104	0.446	**0.905**
HC	−0.364	−0.348	−0.155	−0.196	0.139	−0.517	0.692	0.138	−0.104	−0.611	0.343	1	0.568	−0.077	0.129
FC	−0.358	0.077	−0.687	−0.113	−0.495	−0.46	−0.193	−0.495	−0.47	−0.581	−0.104	0.568	1	**−0.845**	−0.309
HC/FC	0.096	−0.185	**0.836**	0.146	**0.795**	0.38	0.644	**0.795**	0.544	0.403	0.446	−0.077	**−0.845**	1	0.611
CEC	−0.639	0.487	**0.845**	**0.738**	**0.95**	**0.737**	0.432	**0.951**	0.593	0.375	**0.905**	0.129	−0.309	0.611	1

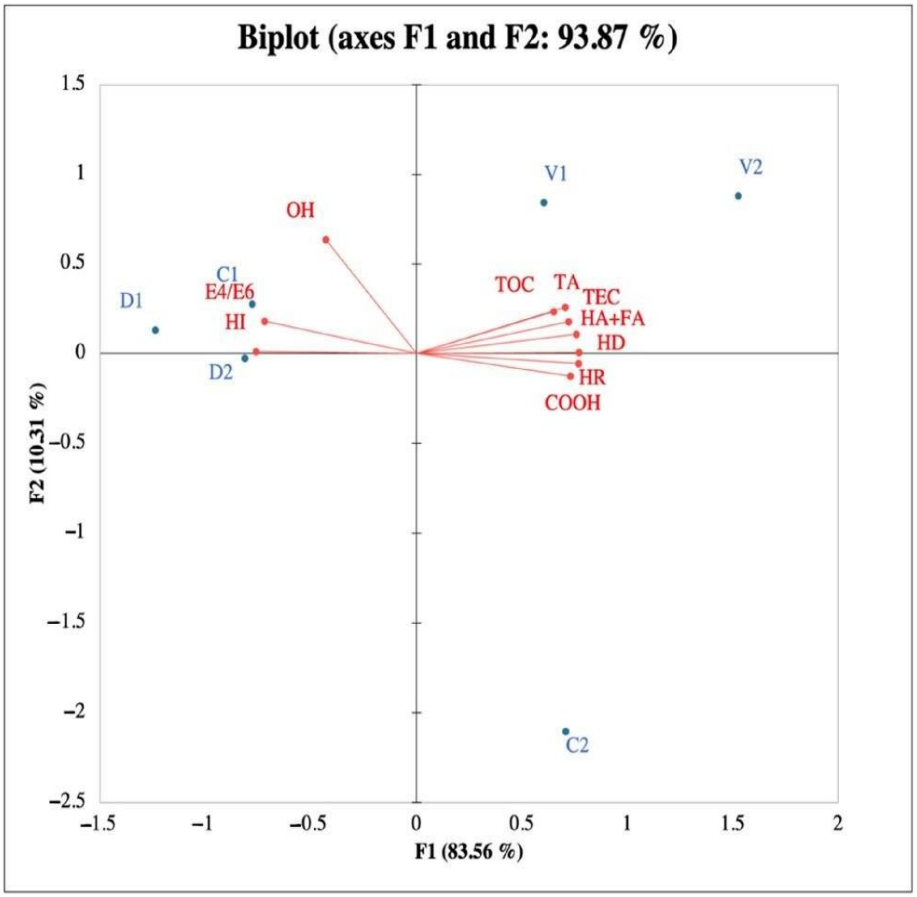


Figure 4. PCA of humic characteristics of C1, C2, D1, D2, V1 and V2.

The results of the E4/E6 ratio suggested a greater degree of aromaticity for the vermicompost than compost and digestate (Table 5). The E4/E6 ratio is inversely related to the degree of condensation, lower is the value higher is the aromaticity degree.

Carboxyl groups were the major component of the total acidity for compost produced with orange waste and vermicompost produced from both olive and orange wastes. Phenolic OH were the greater components of total acidity for both digestates and for compost olive.

The DRIFT spectrum was characterized by broad bands showing great differences among compost vermicompost and digestate. Both vermicompost expressed more peaks than both compost and digestate (Figure 6).

The DRIFT spectra of compost (Figure 6) produced with olive and orange wastes showed mainly the existence of O-containing functional groups. The spectra indicated that compost from orange contained peaks at 1712 cm^{-1} (mostly CO_2H groups), at 1646 (mostly aromatic C=C), and at 1220 (mostly OH or CO_2H groups) distinct and sharp. The high intensity of these bands reflects the high solubility of this HS. These peaks were less prominent in the spectra of compost from olive waste. The peak at 831 to 1013 cm^{-1} (aliphatic C-H) were attributed mainly to C-O stretching of carbohydrates [36], as well as to aromatic CH out of plane bending. Compost C1 had a higher peak at a 1538, indicating an aromatic C=C stretch. The peak near 843 was mainly attributed to aromatic CH out of plane bending. V1 had more peaks mostly V2 coming from orange waste. In addition to the peaks detected in compost distinct and sharp were at 2444 cm^{-1} showing OH stretch of H-bounded in COOH groups. At the peaks at 1660-1645 cm^{-1} present in both vermicompost denoted

the presence of C=O stretching of Amide I, quinones and H bonded conjugated ketones. V2 in respect to V1 contained more peaks attributed mainly to C-O stretching of carbohydrates. Digestate presented less peaks than compost and vermicompost, and spectra indicated COOH groups (1712 cm^{-1}), amide I and II (1646 and 1558 cm^{-1} respectively) COO- symmetric stretching mode (1408 cm^{-1}). Peaks near the amide I (1660 cm^{-1}) were resolved clearly. The band at 1712 cm^{-1} was assigned to the C=O stretching mode of COOH groups [53]. The bands at 1409 only present in digestate D2 was due to CH2 bending and COO−symmetric stretching modes. Strong negative peaks were near, 843, and 822 cm^{-1} they were attributed mainly to C-O stretching of carbohydrates [36], as well as to C-C stretching motions of aliphatic groups and in-plane CH bending of aromatic rings. The band characteristic of aromatic rings generally found at 1514 cm^{-1}, was absent while the presence of that at 1221 cm^{-1} in D1 was due to C-O stretching vibrations in phenols and carboxyl groups.

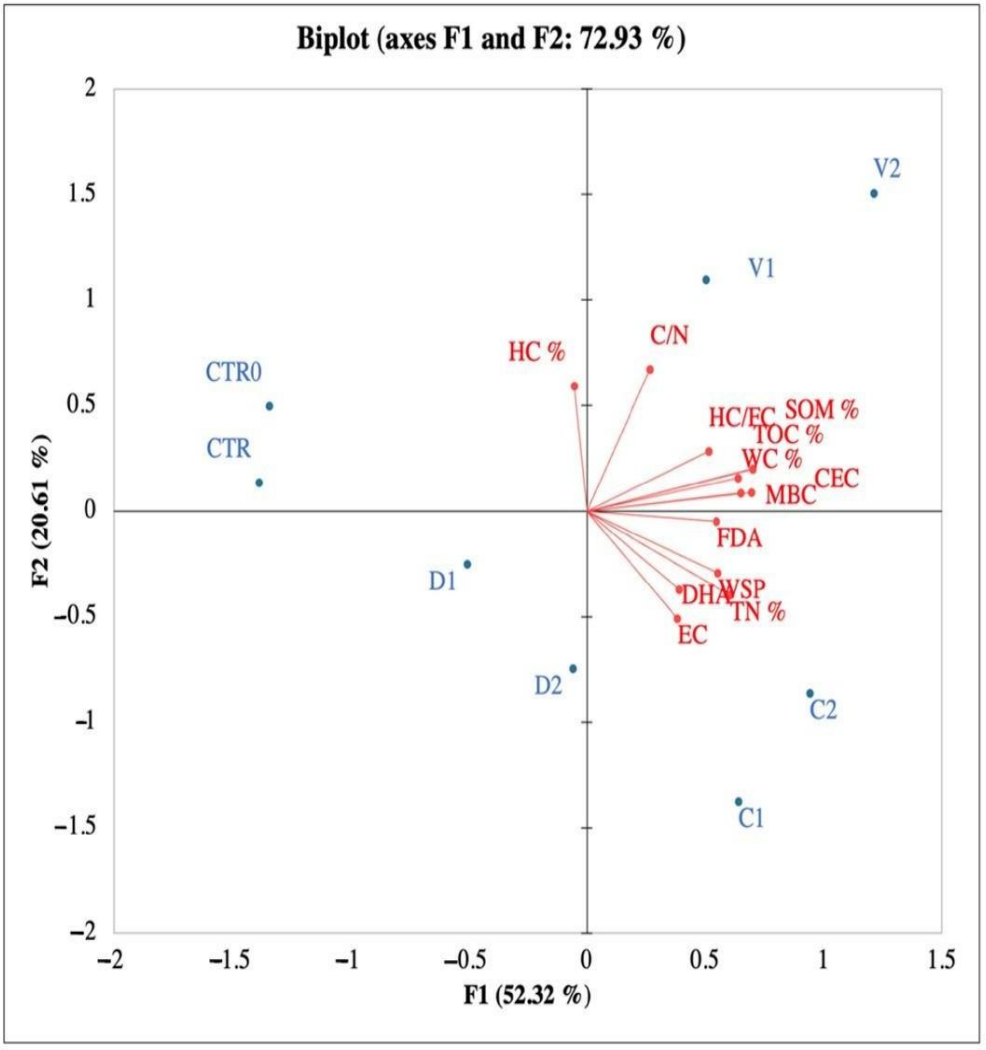


Figure 5. PCA of physical and chemical properties of potted alkaline sandy-loam soils CTR0, not amended soil was used as control (CTR) and six months after the addition of: C1, C2, D1, D2, V1 and V2.

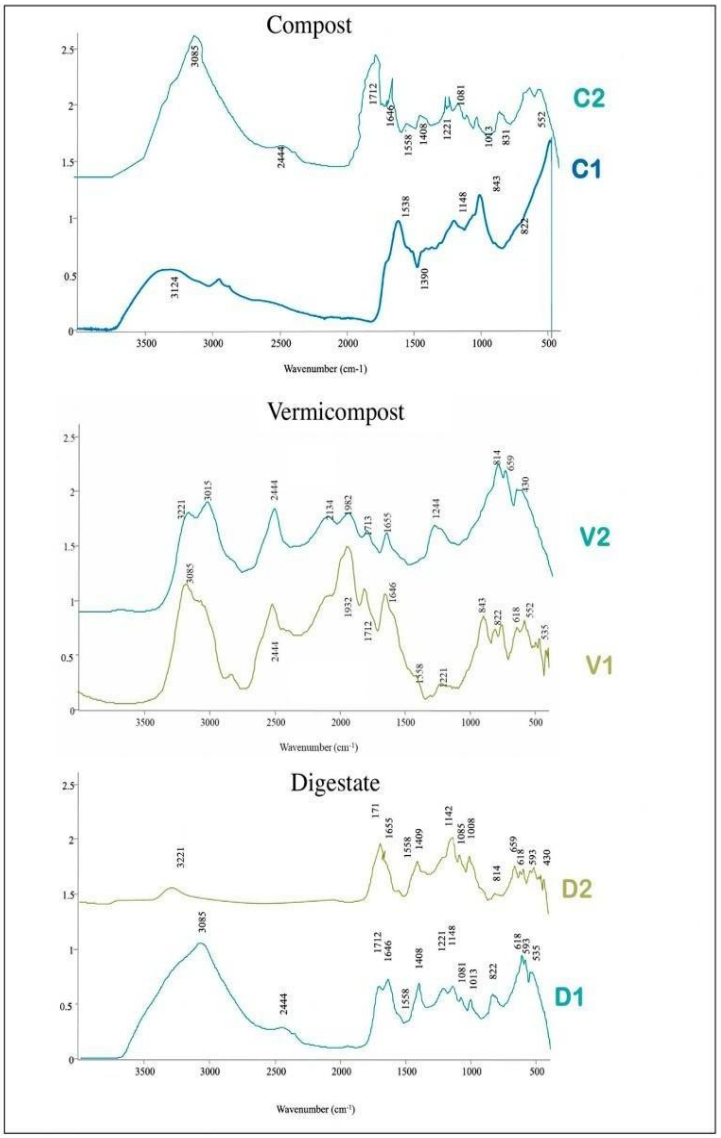


Figure 6. DRIFT spectra of compost C1, C2, D1, D2, V1 and V2.

4. Discussion

Aerobic and anaerobic transformation of organic wastes is a complex process driven by a diverse community of microorganisms, which utilize nitrogen (N) and carbon (C) for their metabolic activities [54,55]. Bacteria, fungi, and actinomycetes are the primary microbial agents driving these transformation processes, each contributing with varying intensity based on multiple factors [56,57]. Critical parameters influencing microbial activity include temperature [58], moisture content, oxygen availability [59], and chemical composition of the raw materials. Our findings highlighted substantial differences in the chemical composition of the two raw materials, which significantly affected transformation processes and microbial dynamics. Additionally, the comparison of different transformation processes showed also that both aerobic methods—composting and vermicomposting—produced fertilizers of better quality compared to digestate obtained through anaerobic processes. Data evidenced that olive wastes underwent to both anaerobic and aerobic transformation processes produced by-compounds richer in water soluble phenols with less carbon and

nutrients such as potassium magnesium, phosphate and sulphate, in respect to the orange by-products. Conversely, orange by-products contained HS with a greater amount of HA+FA, higher humification rate and degree and lower humification index and E4/E6 ratio. Based on the findings that humification index (HI), and humification rate are effective measures to evaluate the extent of humification in organic materials, relating the humification index decrease and the humification rate increase to the maturity of the organic matter [60], our data demonstrated a greater maturity of humic matter in all orange by-products. Additionally, referring to the type of process used, the aerobic processes resulted more appropriate than anaerobic in transforming both raw materials with a less production of reduced compounds, and a more microbial biodiversity involved in the transformation process [61]. Vermicomposting generated bioavailable fulvic acids and smaller quantities of highly active humic acids, promoting rapid nutrient cycling and supporting plant growth. In contrast, composting produced a larger quantity of stable humic acids, which can contribute to long-term soil structure and fertility. Digestate, while yielding fewer humic substances overall, provided low-molecular-weight fulvic acids that can offer immediate nutrient availability when applied to soil. Each of these processes, therefore, delivers a unique profile of humic components, allowing for strategic selection based on specific agricultural or environmental goals. Considering both aerobic processes, vermicomposting resulted to produce the best fertilizer quality with both the matrices. The decrease in E4/E6 ratio with the concomitant increase in the peaks of hydroxy O-H in the humic structures of compost and vermicompost as well as the observed increase in the band characteristic of aromatic rings generally found at 1514 cm^{-1}, and the enhanced stretching vibration of aromatic C=C and C=O (1650 cm^{-1}) also indicated an enhanced oxidation degree of humic substances, and the appearance of C-O-C (1244 cm^{-1}) in vermicompost and compost indicated the high structural stability of humic substances, accompanied by the enhancement of humification degree. This is consistent with the results of the elemental analysis which demonstrated a greater number of carboxylic groups in HS contained in compost and vermicompost. COOH (carboxyl) groups in humic substances provides a clearer understanding of how these functional groups contribute to soil health by enhancing nutrient retention, promoting cation exchange capacity, and fostering beneficial microbial activity. These interactions ultimately improve soil structure, fertility, and resilience, making COOH groups a key factor in the positive effects of humic substances on soil. Data on soil evidenced that nevertheless, the addition of all the by-products improved SOM, MBC and enzymatic activities in respect to control, the greatest increase was observed with both vermicompost, followed in ranking by compost and digestate. A greater cation exchange capacity was also observed in soil treated with vermicompost in respect to the other treatments and this can be related to the greatest COOH groups of humic substances contained into it. The Pearson data evidenced a good correlation of humic characteristics of V1, V2 and C2 with the main indicators of soil quality. It is well known that the earthworms fragment the organic waste substrates, stimulate microbial activity greatly and increase rates of mineralization, rapidly converting the wastes into humus-like substances with a finer structure than composts but possessing a greater and more diverse microbial activities. The consistent improvements in soil quality observed with the use of vermicompost appeared to be primarily due to their humic substance content, rather than changes in nutrient levels. Vermicomposting is a faster transformation process able to accelerate the production of humus, which not only improve soil health but also offer a sustainable alternative to synthetic fertilizers. As reported by Fornes et al. [62] one of the most significant factors affecting the quality of products such as composts and vermicompost is the original raw materials. In this study, emerged that vermicomposting and composting are superior to anaerobic digestion in producing humus-rich fertilizers primarily because of the differences in oxygen availability, microbial activity, and organic matter breakdown during these processes, which directly impact humus formation. Oxygen facilitates the activity of aerobic microorganisms that efficiently decompose organic matter and transform it into humic substances. This oxygen-rich environment enhances humification, the process by which organic matter breaks down into

stable humic substances, which are crucial for soil health. In contrast, anaerobic digestion occurs in oxygen-deprived conditions, which supports anaerobic bacteria but limits the diversity and activity of organisms that contribute to humus formation. Consequently, anaerobic digestion produces fewer humic substances and a less stable organic product, resulting in a fertilizer with lower humus content and reduced soil enhancement properties. The results of this study that compared the characteristics of composts and vermicompost as aerobic by-products and digestate as anaerobic by-product, obtained in parallel, using the same organic wastes processed with the same processes, gave new insight on their use.

5. Conclusions

The study demonstrated that transforming organic wastes, specifically olive pomace and orange waste, through different processing methods significantly alters the chemical and humic composition of the resulting fertilizers, with each method providing distinct advantages for soil health. All tested fertilizers, regardless of substrate or processing technique, exhibited high levels of humic substances (HSs), which positively influenced key soil parameters, including microbial biomass, enzymatic activity, and cation exchange capacity.

Among the methods examined, vermicomposting emerged as the most effective, yielding fertilizers with enhanced humic stability, nutrient retention, and strong associations with improved soil quality indicators. Fertilizers derived from vermicomposted olive pomace and orange wastes were particularly effective in increasing microbial diversity and soil structural stability, reinforcing vermicompost's superior role in promoting long-term soil fertility and health.

In conclusion, the aerobic and anaerobic processing of organic wastes like olive pomace and orange wastes not only reduces landfill burden and greenhouse gas emissions but also reintegrates valuable organic matter into the soil, fostering sustainable agricultural systems. This process offers a viable, eco-friendly alternative to synthetic fertilizers, with potential to enhance soil health and productivity.

Future research should expand the range of organic waste types and assess the long-term impacts on soil health and fertility. Additionally, exploring the influence of varying environmental conditions on transformation processes and microbial communities could provide deeper insights. Investigating the synergistic effects of combining different organic waste types during processing may further improve fertilizer quality and maximize soil health benefits.

Author Contributions: Conceptualization, A.M. (Adele Muscolo); writing—original draft preparation, A.M. (Adele Muscolo) and A.M. (Angela Maffia); statistical analysis, A.M. (Angela Maffia); project administration, A.M. (Adele Muscolo); software, F.C. and F.M.; formal analysis C.M., S.B. and M.O. All authors have read and agreed to the published version of the manuscript.

Funding: This study was carried out in the "Tech4You—Technologies for climate change adaptation and quality of life improvement", PNRR codice identificativo ECS 00000009, CUP: C33C22000290006, (Piano Nazionale di Ripresa e Resilienza (PNRR)-Missione 4, Componente 2, Investimento 1.5 "Creazione e rafforzamento di "ecosistemi dell'innovazione", costruzione di "leader territoriali di R&S" Spoke 3—Goal 3.5.

Data Availability Statement: The original contributions presented in the study are included in the article further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Tripathi, S.; Srivastava, P.; Devi, R.S.; Bhadouria, R. Influence of synthetic fertilizers and pesticides on soil health and soil microbiology. In *Agrochemicals Detection, Treatment and Remediation—Pesticides and Chemical Fertilizers*; Elsevier: Amsterdam, The Netherlands, 2020; pp. 25–54. [CrossRef]
2. Schrama, M.; De Haan, J.J.; Kroonen, M.; Verstegen, H.; Van Der Putten, W.H. Crop yield gap and stability in organic and conventional farming systems. *Agric. Ecosyst. Environ.* **2018**, *256*, 123–130. [CrossRef]

3. Barłóg, P.; Grzebisz, W.; Łukowiak, R. Fertilizers and Fertilization Strategies Mitigating Soil Factors Constraining Efficiency of Nitrogen in Plant Production. *Plants* **2022**, *11*, 1855. [CrossRef] [PubMed]
4. Thanigaivel, S.; Vinayagam, S.; Gnanasekaran, L.; Suresh, R.; Soto-Moscoso, M.; Chen, W.-H. Environmental fate of aquatic pollutants and their mitigation by phycoremediation for the clean and sustainable environment: A review. *Environ. Res.* **2023**, *240*, 117460. [CrossRef] [PubMed]
5. Rani, M.; Kaushik, P.; Bhayana, S.; Kapoor, S. Impact of organic farming on soil health and nutritional quality of crops. *J. Saudi Soc. Agric. Sci.* **2023**, *22*, 560–569. [CrossRef]
6. Costa, C.; García-Lestón, J.; Costa, S.; Coelho, P.; Silva, S.; Pingarilho, M.; Valdiglesias, V.; Mattei, F.; Dall'Armi, V.; Bonassi, S.; et al. Is organic farming safer to farmers' health? A comparison between organic and traditional farming. *Toxicol. Lett.* **2014**, *230*, 166–176. [CrossRef]
7. Ahmad, M.F.; Ahmad, F.A.; Alsayegh, A.A.; Zeyaullah, M.; AlShahrani, A.M.; Muzammil, K.; Saati, A.A.; Wahab, S.; Elbendary, E.Y.; Kambal, N.; et al. Pesticides impacts on human health and the environment with their mechanisms of action and possible countermeasures. *Heliyon* **2024**, *10*, e29128. [CrossRef]
8. Alengebawy, A.; Abdelkhalek, S.T.; Qureshi, S.R.; Wang, M.-Q. Heavy Metals and Pesticides Toxicity in Agricultural Soil and Plants: Ecological Risks and Human Health Implications. *Toxics* **2021**, *9*, 42. [CrossRef]
9. Tripathi, A.D.; Mishra, R.; Maurya, K.K.; Singh, R.B.; Wilson, D.W. Estimates for world population and global food availability for global health. In *The Role of Functional Food Security in Global Health*; Academic Press: Cambridge, MA, USA, 2018; pp. 3–24. [CrossRef]
10. Lou, X.F.; Nair, J. The impact of landfilling and composting on greenhouse gas emissions—A review. *Bioresour. Technol.* **2009**, *100*, 3792–3798. [CrossRef]
11. PROSODOL. Presented at the Olive Oil Mills Wastes and Environmental Protection Symposium, Chania, Greece, October 2012; pp. 16–18. Available online: https://www.prosodol.gr/ (accessed on 12 June 2024).
12. Panuccio, M.R.; Marra, F.; Maffia, A.; Muscolo, A. Recycling of agricultural (orange and olive) bio-wastes into ecofriendly fertilizers for improving soil and garlic quality. *Resour. Conserv. Recycl. Adv.* **2022**, *15*, 200083. [CrossRef]
13. Hajam, Y.A.; Kumar, R.; Kumar, A. Environmental waste management strategies and vermi transformation for sustainable development. *Environ. Chall.* **2023**, *13*, 100747. [CrossRef]
14. Maffia, A.; Marra, F.; Celano, G.; Oliva, M.; Mallamaci, C.; Hussain, M.I.; Muscolo, A. Exploring the Potential and Obstacles of Agro-Industrial Waste-Based Fertilizers. *Land* **2024**, *13*, 1166. [CrossRef]
15. Silva, M.E.F.; De Lemos, L.T.; Nunes, O.C.; Cunha-Queda, A.C. Influence of the composition of the initial mixtures on the chemical composition, physicochemical properties and humic-like substances content of composts. *Waste Manag.* **2013**, *34*, 21–27. [CrossRef] [PubMed]
16. Kumar, M.; Ou, Y.-L.; Lin, J.-G. Co-composting of green waste and food waste at low C/N ratio. *Waste Manag.* **2010**, *30*, 602–609. [CrossRef] [PubMed]
17. Bhatia, A.; Madan, S.; Sahoo, J.; Ali, M.; Pathania, R.; Kazmi, A.A. Diversity of bacterial isolates during full scale rotary drum composting. *Waste Manag.* **2013**, *33*, 1595–1601. [CrossRef]
18. Jurado, M.M.; Suárez-Estrella, F.; López, M.J.; Vargas-García, M.C.; López-González, J.A.; Moreno, J. Enhanced turnover of organic matter fractions by microbial stimulation during lignocellulosic waste composting. *Bioresour. Technol.* **2015**, *186*, 15–24. [CrossRef]
19. Manzoor, A.; Naveed, M.S.; Ali, R.M.A.; Naseer, M.A.; Ul-Hussan, M.; Saqib, M.; Hussain, S.; Farooq, M. Vermicompost: A potential organic fertilizer for sustainable vegetable cultivation. *Sci. Hortic.* **2024**, *336*, 113443. [CrossRef]
20. Klassen, V.; Blifernez-Klassen, O.; Wobbe, L.; Schlüter, A.; Kruse, O.; Mussgnug, J.H. Efficiency and biotechnological aspects of biogas production from microalgal substrates. *J. Biotechnol.* **2016**, *234*, 7–26. [CrossRef]
21. Guo, X.-X.; Liu, H.-T.; Wu, S.-B. Humic substances developed during organic waste composting: Formation mechanisms, structural properties, and agronomic functions. *Sci. Total Environ.* **2019**, *662*, 501–510. [CrossRef]
22. Ampong, K.; Thilakaranthna, M.S.; Gorim, L.Y. Understanding the role of humic acids on crop performance and soil health. *Front. Agron.* **2022**, *4*, 848621. [CrossRef]
23. Loffredo, E.; Senesi, N. In vitro and in vivo assessment of the potential of compost and its humic acid fraction to protect ornamental plants from soil-borne pathogenic fungi. *Sci. Hortic.* **2009**, *122*, 432–439. [CrossRef]
24. Mehta, C.M.; Palni, U.; Franke-Whittle, I.H.; Sharma, A.K. Compost: Its role, mechanism and impact on reducing soil-borne plant diseases. *Waste Manag.* **2013**, *34*, 607–622. [CrossRef] [PubMed]
25. Hu, Z.-T.; Huo, W.; Chen, Y.; Zhang, Q.; Hu, M.; Zheng, W.; Shao, Y.; Pan, Z.; Li, X.; Zhao, J. Humic substances derived from biomass waste during aerobic composting and hydrothermal treatment: A review. *Front. Bioeng. Biotechnol.* **2022**, *10*, 878686. [CrossRef] [PubMed]
26. Long, S.; Yang, J.; Hao, Z.; Shi, Z.; Liu, X.; Xu, Q.; Wang, Y.; Wang, D.; Ni, B.-J. Multiple roles of humic substances in anaerobic digestion systems: A review. *J. Clean. Prod.* **2023**, *418*, 138066. [CrossRef]
27. Lanno, M.; Klavins, M.; Purmalis, O.; Shanskiy, M.; Kisand, A.; Kriipsalu, M. Properties of humic substances in composts comprised of different organic source material. *Agriculture* **2022**, *12*, 1797. [CrossRef]
28. Liang, C.; Das, K.C.; McClendon, R.W. The Influence of Temperature and Moisture Contents Regimes on the Aerobic Microbial Activity of a Biosolids Composting Blend. *Bioresour. Technol.* **2003**, *86*, 131–137. [CrossRef]

29. ANPA (Agenzia Nazionale per la Protezione dell'Ambiente). Metodi di analisi del compost. In *Manuali e Linee Guida*; ISPRA: Rome, Italy, 2001; Volume 3.
30. Box, J.D. Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
31. Wang, Z.; Gao, M.; Wang, Z.; She, Z.; Hu, B.; Wang, Y.; Zhao, C. Comparison of physicochemical parameters during the forced-aeration composting of sewage sludge and maize straw at different initial C/N ratios. *J. Air Waste Manag. Assoc.* **2013**, *63*, 1130–1136. [CrossRef]
32. Sommer, S.G.; Kjellerup, V.; Kristjansen, O. Determination of total ammonium nitrogen in pig and cattle slurry: Sample preparation and analysis. *Acta Agric. Scand. Sect. B—Soil Plant Sci.* **1992**, *42*, 146–151. [CrossRef]
33. Nardi, S.; Pizzeghello, D.; Reniero, F.; Rascio, N. Chemical and Biochemical Properties of Humic Substances Isolated from Forest Soils and Plant Growth. *Soil Sci. Soc. Am. J.* **2000**, *64*, 639–645. [CrossRef]
34. Muscolo, A.; Sidari, M. Carboxyl and phenolic humic fractions affect *Pinus nigra* callus growth and metabolism. *Soil Sci. Soc. Am. J.* **2009**, *73*, 1119–1129. [CrossRef]
35. Bellamy, J.L. *The Infrared Spectra of Complex Molecules*; Chapman & Hall: London, UK, 1975.
36. Stevenson, F.J. *Humus Chemistry: Genesis, Composition, Reactions*, 2nd ed.; Wiley: New York, NY, USA, 1994.
37. Francioso, O.; Sánchez-Cortés, S.; Casarini, D.; Garcia-Ramos, J.V.; Ciavatta, C.; Gessa, C. Spectroscopic study of humic acids fractionated by means of tangential ultrafiltration. *J. Mol. Struct.* **2002**, *609*, 137–147. [CrossRef]
38. Schnitzer, M.; Khan, S.U. *Humic Substances in the Environment*; CABI: Wallingford, UK, 1972.
39. Perdue, E.M. *Acidic Functional Groups of Humic Substances*; CABI: Wallingford, UK, 1985; pp. 493–526.
40. Ciavatta, C.; Govi, M.; Antisari, L.V.; Sequi, P. Characterization of humified compounds by extraction and fractionation on solid polyphnilpyrrolidone. *J. Chromatogr.* **1990**, *509*, 141–146. [CrossRef]
41. FAO. *Methods of Analysis for Soils of Arid and Semi-Arid Regions*; Food and Agricultural Organization: Rome, Italy, 2007; p. 57.
42. Maffia, A.; Marra, F.; Battaglia, S.; Oliva, M.; Mallamaci, C.; Muscolo, A. Influence of Agro-Industrial Waste Composts on Soil Characteristics, Growth Dynamics, and Yield of Red Cabbage and Broccoli. *Soil Syst.* **2024**, *8*, 53. [CrossRef]
43. Muscolo, A.; Papalia, T.; Settineri, G.; Mallamaci, C.; Jeske-Kaczanowska, A. Are raw materials or composting conditions and time that most influence the maturity and/or quality of composts? Comparison of obtained composts on soil properties. *J. Clean. Prod.* **2018**, *195*, 93–101. [CrossRef]
44. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 2005.
45. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
46. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoff in organishen Kopern. *Anal. Chem.* **1883**, *22*, 354–358.
47. Bragança, A.; Fernandes, R.; de Carvalho Junior, I.A.; Silva Ribeiro, J.E.; de Sá Mendonça, E. Comparison of different methods for the determination of total organic carbon and humic substances in Brazilian soils. *Rev. Ceres* **2015**, *62*, 496–501. Available online: https://www.redalyc.org/articulo.oa?id=305241509011. (accessed on 4 July 2024).
48. Mehlich, A. Rapid Determination of Cation and Anion Exchange Properties and pHe of Soils. *J. Assoc. Off. Agric. Chem.* **1953**, *36*, 445–457. [CrossRef]
49. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951. [CrossRef]
50. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
51. Von Mersi, W.; Schinner, F. An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
52. *AS 3741-1990*; Recommended Practice for Chemical Analysis by Ion Chromatography. Standards Australia: Sidney, Australia, 1990.
53. Niemeyer, J.; Chen, Y.; Bollag, J.-M. Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. *Soil Sci. Soc. Am. J.* **1992**, *56*, 135–140. [CrossRef]
54. Zhao, X.; Li, J.; Che, Z.; Xue, L. Succession of the bacterial communities and functional characteristics in sheep manure composting. *Biology* **2022**, *11*, 1181. [CrossRef] [PubMed]
55. Niya, B.; Yaakoubi, K.; Beraich, F.Z.; Arouch, M.; Kadmiri, I.M. Current status and future developments of assessing microbiome composition and dynamics in anaerobic digestion systems using metagenomic approaches. *Heliyon* **2024**, *10*, e28221. [CrossRef]
56. Rastogi, M.; Nandal, M.; Khosla, B. Microbes as vital additives for solid waste composting. *Heliyon* **2020**, *6*, e03343. [CrossRef]
57. Palaniveloo, K.; Amran, M.A.; Norhashim, N.A.; Mohamad-Fauzi, N.; Fang, P.; Low, H.; Yap, K.; Looi, J.; Chian-Yee, M.G.; Lai, J.; et al. Food waste composting and microbial community structure profiling. *Processes* **2020**, *8*, 723. [CrossRef]
58. Cesaro, A.; Conte, A.; Belgiorno, V.; Siciliano, A.; Guida, M. The evolution of compost stability and maturity during the full-scale treatment of the organic fraction of municipal solid waste. *J. Environ. Manag.* **2018**, *232*, 264–270. [CrossRef]
59. Lin, C.; Cheruiyot, N.K.; Bui, X.-T.; Ngo, H.H. Composting and its application in bioremediation of organic contaminants. *Bioengineered* **2022**, *13*, 1073–1089. [CrossRef]
60. Ciavatta, C.; Govi, M.; Antisari, L.V.; Sequi, P. An enzymatic approach to the determination of the degree of stabilization of organic carbon in fertilizers. *Fertil. Res.* **1990**, *25*, 167–174. [CrossRef]

61. Mehta, C.; Sirari, K. Comparative study of aerobic and anaerobic compostingfor better understanding of organic waste management: A mini review. *Plant Arch.* **2018**, *18*, 44–48.
62. Fornes, F.; Mendoza-Hernandez, D.; Belda, R.M. Compost versus vermicompost as substrate constituents for rooting shrub cuttings. *Span. J. Agric. Res.* **2013**, *11*, 518–528. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

Exploring the Potential and Obstacles of Agro-Industrial Waste-Based Fertilizer

Angela Maffia [1], Federica Marra [1] Muhammad Iftikhar Hussain [3], Giuseppe Celano [2] Mariateresa Oliva[1], Carmelo Mallamaci [1] and AdeleMuscolo[1]*

[1] Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy;
[2] Department of FARMACIA, Course of Agriculture, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy.
[3] Department of Plant Biology & Soil Science, Universidad de Vigo, Campus Lagoas Marcosende, 36310 Vigo, Spain.

*Correspondence: amuscolo@unirc.it; Tel.: +39-0965169436

Land

https://doi.org/10.3390/land13081166

Received: 24 June 2024
Revised: 19 July 2024
Accepted: 25 July 2024
Published: 29 July 2024

land

MDPI

Article

Exploring the Potential and Obstacles of Agro-Industrial Waste-Based Fertilizers

Angela Maffia [1], Federica Marra [1], Giuseppe Celano [2], Mariateresa Oliva [1], Carmelo Mallamaci [1], Muhammad Iftikhar Hussain [3] and Adele Muscolo [1,*]

[1] Department of AGRARIA, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy; angela.maffia@unirc.it (A.M.); federica.marra@unirc.it (F.M.); mariateresa.oliva@unirc.it (M.O.); carmelo.mallamaci@unirc.it (C.M.)

[2] Department of FARMACIA, Course of Agriculture, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy; gcelano@unisa.it

[3] Department of Plant Biology & Soil Science, Universidad de Vigo, Campus Lagoas Marcosende, 36310 Vigo, Spain; iftikhar@uvigo.es

* Correspondence: amuscolo@unirc.it; Tel.: +39-09651694364

Abstract: Conducted in Southern Italy's Calabria region, this study aimed to repurpose olive wastes, which are still a source of valuable biomolecules including plant nutrients, flavonoids, polysaccharides, and phenolic compounds, into compost to be used in sustainable agriculture as fertilizers, in alternative to synthetic substances. The compost underwent chemical analysis and soil fertility testing to support eco-friendly agricultural practices. Factors like extraction process, waste composition, and percentage of waste in composting were studied for their impact. The research evaluated compost fertilizing effectiveness by analyzing soil chemical and biological properties 180 days after the application. The results demonstrated that the proportion of olive oil waste and the olive oil extraction method significantly impacted compost quality and its environmental footprint. All composts improved soil properties but to a different extent. Compost olive waste 3 (OWC3; 34% olive oil waste, 33% buffalo manure, and 33% straw) was the most effective in enhancing soil fertility. Compost olive waste 1 (OWC1), with the same olive waste percentage as compost olive waste 2 (OWC2) but from a different extraction process, outperformed OWC2 in enhancing soil fertility and microbial activity. The research highlighted the importance of organic matter addition to soil and the significant role of both raw material percentage and extraction process in compost quality. Life cycle assessment indicated that OWC3 had the lowest environmental impact and the highest fertilizing power. Composting represents a practical way to manage organic wastes and improve soil quality, providing essential nutrients for soil health and ecosystem functioning.

Keywords: olive pomace; compost; soil fertility; life cycle assessment; sustainability

check for updates

Citation: Maffia, A.; Marra, F.; Celano, G.; Oliva, M.; Mallamaci, C.; Hussain, M.I.; Muscolo, A. Exploring the Potential and Obstacles of Agro-Industrial Waste-Based Fertilizers. *Land* **2024**, *13*, 1166. https://doi.org/10.3390/land13081166

Academic Editor: Dionisios Gasparatos

Received: 24 June 2024
Revised: 19 July 2024
Accepted: 25 July 2024
Published: 29 July 2024

Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

The frequent or unregulated application of synthetic chemicals like nitrogen, phosphorus, and potassium to enhance plant growth leads to the deterioration of soil fertility and quality worldwide, a heightened risk of diseases due to the diminished nutritional and edible value of crops, and adverse impacts on human nutrition and health due to the low concentration of protein and micronutrients (e.g., Zn, Fe, Se, B, I) in crops, aggravating malnutrition, which affects 3.7 billion people, especially children [1,2]. Particularly, Mediterranean soils are under significant pressure due to various environmental (extreme events and climatic changes) and anthropogenic factors (unsustainable agriculture and land use transformation) [3]. Agricultural intensification in these regions is the major contributor to soil degradation, leading to substantial losses in soil organic carbon (SOC) and increased erosion. Intensive farming practices with heavy use of chemical fertilizers (urea, diammonium phosphate and potassium chloride, potassium nitrate, NPK) and pesticides

(chlorpyrifos, carbendazim, alphametrin, dichlorvos) are accelerating the mineralization of organic matter, disrupting soil structure and decreasing soil biodiversity [4]. This not only depletes SOC but also reduces the soil's ability to retain water and nutrients, making it more susceptible to erosion and degradation [5]. Moreover, the Mediterranean climate, with ever-increasingly hot, dry summers and mild, wet winters, is exacerbating these issues [6]. As a result, the soil's fertility and productivity have declined over time, posing a threat to agriculture. The agricultural sector faces a dual challenge: the need to enhance crop productivity to meet the growing global food demand and the imperative to adopt sustainable practices that mitigate environmental degradation. The by-products of various agricultural and food processing activities represent a promising yet underutilized resource for addressing these challenges [7]. By transforming these wastes into fertilizers, it is potentially possible to reduce dependency on synthetic fertilizers, reduce waste disposal issues, and promote a circular economy. It is essential to adopt practices that enhance SOC levels and improve soil quality. These practices include the use of organic amendments to protect the soil from erosion, enhance biodiversity, and improve overall ecosystem resilience [8]. According to the latest update from the European Commission, Italian olive oil production reached 324,000 tons in the 2023/24 crop year, 80 percent of which is located in southern Italy, where Puglia represents the most important region, with about 370,000 ha, followed by Calabria (about 186,000 ha) and Sicily (about 160,000 ha) [9]. The olive oil extraction industry is a key agro-industrial sector in Mediterranean countries, playing a significant role in their economic and social development. Due to its nutritional benefits and rich bioactive compounds such as flavonoids, polyphenols, anthocyanins, and organic matter, olive oil remains a highly sought-after food product globally, with demand continually rising [10]. The extraction of olive oil can be performed using traditional pressing methods, three-phase centrifugal systems, or two-phase centrifugal systems. All these methods generate substantial amounts of waste and by-products. The extraction of one metric ton of olive oil using three-phase systems results in approximately 0.6 tons of olive solid waste (OSW) and around 1.5 cubic meters of olive wastewater (OWW) [11]. The two-phase process generates a semi-solid waste with higher moisture content [12], reducing the amount of olive wastewater. Olive solid residues were once deemed undesirable due to their environmental impact and the significant costs associated with their management and disposal concentrated in short period of production (October–November). These wastes are in the category of highly polluting and phytotoxic materials according to EU Waste Framework Directive (2008/98/EC) [13], but their richness in valuable compounds, such as organic matter, potassium and water-soluble carbohydrates, can be considered a potential to be exploited with the promotion of the new approach of a circular economy. Numerous studies have indicated that olive wastes, even if they are polluting, mainly related to phenolic compounds with allelopathic and phytotoxic effects, can be used raw or composted due to their high content of organic matter and nutrients to improve soil fertility, closing the residue–resource cycle [14–16]. The type and quantity of by-products and the way they are used can diversely affect key soil properties and crop responses [17]. Vignozzi et al. [18] in Mediterranean agro-ecosystems used by-products of the olive oil industry as organic amendments in comparison to organo-mineral fertilizer. Their results evidenced that the composted olive wastes induced the largest increase in soil TOC, TEC, and HC content. Compost and co-composts derived from olive mill wastes (OMWs) have been effectively used as organic fertilizers for horticultural crops [16], olive trees [19], and also as part of the substrate or growing media for ornamental plant culture [20]. Alfano et al. [21] also provided evidence of the disease-suppressive effect of compost from olive mill residues on several soil-borne plant pathogens. López-Piñeiro et al. [22], in a study conducted in Spain on an olive plantation, evidenced significant increases in organic carbon, total N, available P and K, and aggregate stability in olive-compost-amended soils. Leaf analysis of olive trees showed significant increases in N, P, and K concentrations in treated plots after the two first years of olive compost amendments. Also, a general increase in olive production was observed in the treated plots. On the basis of previous mentioned

results, this manuscript delves into the potential and obstacles associated with the use of olive waste-based fertilizers. Despite the promising prospects, several obstacles hinder the widespread adoption of olive waste-based fertilizers. These include technical challenges in waste conversion processes, regulatory hurdles, market acceptance, and the need for comprehensive environmental impact assessments.

By overcoming these challenges, we can establish a foundation for more sustainable agricultural methods that align with global environmental objectives. Given the vital role of the olive sector in the Mediterranean region from socio-economic and cultural standpoints, particularly in relation to the healthy Mediterranean Diet, it is crucial to address the issue of olive waste, known for its rich content of phenolic compounds, fatty acids, tannins, and other pollutants that are detrimental to the agri-food production chain. Our research hypothesis posited that through composting processes, it is feasible to transform olive waste into a stabilized, sanitized product suitable for use as organic fertilizers. The primary objective of this study was to utilize olive waste, sourced from various oil extraction methods, to create compost for agricultural purposes. Prior to application, the composts underwent chemical analysis to ensure compliance with the marketability standards outlined in current Italian regulations (Legislative Decree no. 75/2010) and were tested for their impact on soil fertility and quality. Our aims were (1) to promote a more resilient and environmentally friendly agricultural system that maintains soil fertility by substituting chemical fertilizers with organic waste-derived alternatives, (2) to identify and select cost-effective soil quality indicators for highlighting eco-friendly agricultural practices, and (3) to investigate the factors affecting the quality and efficacy of compost obtained from the extraction process, the chemical composition of waste materials, and the proportion of olive waste utilized in composting. Additionally, we aimed to assess the effectiveness of compost as a soil amendment by monitoring changes in soil chemical and biochemical properties 180 days post-application, as well as to evaluate the overall impact of compost on soil and the environment using the life cycle assessment (LCA) methodology.

2. Materials and Methods

2.1. Raw Materials

The raw organic materials used for composting were olive waste collected in November 2022 from the traditional three-phase olive oil extraction process and from the two-phase centrifugation olive mill Decanter Multi Filter process (DMF) as well as straw as a structuring material. OWC1 consisted of 90% waste from olive oil (pulp and kernels of olives) + 10% straw; OWC2 consisted of 90% waste from olive oil + 10% straw; OWC3 consisted of 34% olive oil waste from the two-phase centrifugation olive mill Decanter process + 33% buffalo manure and 33% straw. The percentage of olive wastes to be used were derived from the results of previous composting experiments to evaluate the maximum olive waste percentage that can be composted (data not published).

2.2. Procedure for Compost Production

Three different composts were produced in separate bins of 300 liters. The mixtures consisted of 90% of olive oil waste (pulp and kernels of olives) from the three-phase olive oil extraction process + 10% straw for OWC1, 90% olive oil waste from a two-phase centrifugation olive mill (DMF) + 10% straw for OWC2, and 34% olive oil waste from a two-phase centrifugation olive mill Decanter + 33% buffalo manure and 33% straw for OWC3. The composting procedure was carried out three times, with the following settings: an initial mesophilic phase at 29 °C lasting 8 days, followed by a thermophilic phase at 50 °C for 20 days, and concluding with a mesophilic phase at 27 °C for 92 days. The rise in temperature resulted from vigorous microbial activity and adequate aeration, which supplied enough oxygen to boost biological activity and sustain aerobic conditions [23]. The subsequent steady temperature of 27 °C was due to reduced microbial activity and the diminished availability of organic material for decomposition. Throughout the process, moisture levels were kept at 50%, and oxygen levels exceeded 15%. These parameters were

monitored daily with a probe placed in the center of the compost mass. Water was added as needed to maintain the 50% moisture level. To ensure aerobic conditions and facilitate decomposition into stable humus, the mixtures were turned daily, keeping the oxygen level above 15%. Complete decomposition and material stabilization were achieved within four months. The compost was then air-dried, crushed to pass through a 2 mm sieve, and thoroughly homogenized. The chemical analysis of the raw materials used for composting (Table 1) showed bulk density values ranging from 461 to 699 kg/m^3. The highest bulk density (699 kg/m^3) was observed in raw materials from the DMF oil production system, while the lowest (461 kg/m^3) was found in broadleaf residues. The C/N ratio was highest in olive wastes from the DMF oil production system and lowest in broadleaf residues.

Table 1. Inventory analyses of production of OWC1, OWC2 and OWC3.

	OWC1	OWC2	OWC3
Oil mill			
Electricity (kWh)	154	100	53
Water	86	76	45
Collection of crop residues			
Straw (kg)	100	100	330
Olive pomace	900	900	340
Manure (kg)			330
Machinery (h)	0.5	0.5	0.8
Diesel and lubricant (MJ)	3.54	3.54	4.98
Electricity (kWh)	1	1	1.24
Human labor (h)	1	1	1.1
Transport			
Machinery (h)	2	2	3
Diesel and lubricant (MJ)	196	196	213
Human labor (h)	1	1	2
Composting process			
Water (m^3)	34	13	23
Machinery (h)	8	8	7
Diesel and lubricant (MJ)	267	267	213
Electricity (kWh)	17	17	15
Human labor (h)	6	6	4
Trasport of the compost and its distribution on the field			
Machinery (h)	4	4	4
Diesel and lubricant (MJ)	426	426	426
Human labor (h)	5	5	5

2.3. Chemical Analysis of Compost

Chemical characterization of the initial waste materials and resulting composts was conducted following the methodologies outlined in the National Agency for Environmental Protection guidelines (ANPA) [24]. The rate of organic matter mineralization was determined by assessing the reduction in organic matter over time. Organic matter loss was calculated using the following formula:

$$\text{Organic matter loss} = (\text{initial mass of carbon} - \text{final mass of carbon/initial mass of carbon}) \times 100$$

Fluorescein diacetate hydrolase (FDA) activity was measured using the method described by Adam and Duncan [25]. The absorbance was measured at 490 nm using a Shimadzu UV/Vis 1800 spectrophotometer. The enzyme activity was expressed as mg of fluorescein released per gram of dry compost [26]. Dehydrogenase activity (DHA) was determined as per the method of von Mersi and Schinner [27] and absorbance was measured at 490 nm. Water-soluble phenols (WSPs) were extracted from compost with water (1:10) and quantified using the Folin–Ciocalteu reagent [28], with tannic acid as standard. Compost samples were extracted with bi-distilled water (compost to water ratio of 1:10) at 25 °C for 24 h to determine ion concentrations via ion chromatography (Dionex ICS-1100,

Thermo Fisher Scientific, Milan, Italy) [29]. Cation exchange capacity (CEC) was determined using an aqueous $BaCl_2$ solution buffered to pH 7.0 to saturate the soil exchange complexes, as described by Mehlich et al. [30]. The maturity of composts was assessed by calculating the germination index of *Cucumis sativus* L seeds [31]. The GI (germination index) combines measures of relative seed germination (%) and relative root elongation (%) to estimate compost toxicity because germination and root elongation are considered the most sensitive parameters, capable of detecting low levels of toxicity which affect the root growth, as well as high toxicity levels which affect the germination [32]. Values higher than 60% indicate the non-phytotoxicity of compost [33].

Anions including nitrate (NO_3) and cations including ammonium (NH_4^+) were detected as reported in Muscolo et al. [29] by ion chromatography, using a chromatography system (Dionex ICS-1100). The ammonium N (NH_4^+-N) was determined according to the 920.03 A.O.A.C. method [34]. Briefly, OFs (organic fractions) were extracted with 1 M potassium chloride (KCl) at a 1:50 mass-to-volume ratio for 2 h; then, an aliquot of filtrate (20 mL) was dispensed into a digestion tube and analyzed in the Kjeldahl automatic instrument after adding 1 g of magnesium oxide (MgO). It is hereby established that nitrate N (NO_3^--N) was determined with the same method, after reduction with 0.5 g of Devarda alloy (Carlo Erba, Milan, Italy). Organic nitrogen is determined by subtracting the ammonium and nitrate nitrogen (an optional test) from the total nitrogen. However, since nitrate nitrogen levels are generally very low, total nitrogen minus ammonium nitrogen will give a good estimate of organic nitrogen in most composts. The ON/TN ratio was mathematically calculated (Francisco da Silva et al., 2020) [35].

2.4. Analysis of Soil and Treatments

The experiments were conducted in an open field in Motta San Giovanni, Loc. Liso, Italy (x: 561023,1; y: 4204908,9; WGS 84 UTM Zone 33 N), where the soil is of the sandy loam (11.85% clay, 23.21% silt, and 64.94% sand) textural class according to FAO soil classification system [36]. The soils are slightly alkaline (pH 8.5) with total organic carbon and nitrogen content of 3.0% and 0.18%, respectively. Soils were divided in plots of 1 m square each and fertilized. Each compost was used in a quantity equivalent to 4.3 quintals per hectare. Each treatment was replicated six-fold. Unfertilized plots were used as control. The experiment was arranged in a randomized complete block design, with 3 parcels for each treatment. The experiments lasted six months and the results are the average of three independent experiments. During the experiment, the plots were irrigated to keep 70% of the field capacity for the vitality of soils; soil water content was monitored through a direct read soil pH/moisture meter—R181 (Hanna Instruments, Woonsocket, RI, USA). The soil samples were air-dried and sieved at 2 mm for chemical analysis, while fresh soil samples, also sieved at 2 mm, were utilized for microbiological analysis. The water content of the soil was determined on a gravimetric basis [37]. The corresponding equation related to the increase in the water-holding capacity of the treated soil over time was calculated. Particle size analysis was conducted using Bouyoucos' method [38]. Dry matter content was determined by weighing samples after drying them for 24 h at 105 °C as described in Muscolo et al. [39]. The pH and electrical conductivity (EC) were measured according to the procedure described by Muscolo et al. [39]. Total organic carbon (TOC) content was determined using the Walkley–Black oxidimetric method [40], while total nitrogen (TN) was detected using the Kjeldahl digestion procedure [41], involving sulfuric acid at 380 °C.

Microbial biomass carbon (MBC) was quantified via the chloroform fumigation–extraction method outlined by Vance et al. [42], using field-moist samples equivalent to 20 g dry weight. Both fumigated and unfumigated soil extracts were analyzed for soluble organic carbon using the Walkley–Black method [40]. MBC was estimated by calculating the difference between the organic carbon extracted from fumigated and unfumigated soils, applying an extraction efficiency coefficient of 0.38 to convert soluble carbon into biomass carbon [42].

Microbial populations were extracted using the method described by Insam and Goberna [43]. Soil samples (2 g) were mixed with 30 glass beads and 20 mL of 0.90%

Sodium Chloride (NaCl), then shaken at 4 °C for 1 h at 12,000× *g* to separate bacteria from solid particles. The supernatant was diluted with sterile one-fourth-strength Ringer solution to standardize the inoculum density. The soil bacterial population was estimated using Waksman's method [44] with nutrient agar medium at a dilution of 10^5. The fungal population was estimated using the dilution plate method [45] with Martin's Rose Bengal agar medium at a dilution of 10^3. The activities of FDA, DHA, ion concentrations, and CEC were determined as described in Section 2. All analyses were performed in triplicate.

2.5. Environmental Impact Assessment

The environmental impact analysis was conducted using the life cycle assessment methodology, in accordance with ISO standards 14040 [46] and 14044 [47]. System boundaries were defined to determine what to include in the environmental impact assessment. The objective of the study was to compare the environmental impacts of three types of compost (OWC1, OWC2 and OWC3). The system boundaries and the input and output flows considered are depicted in Figure 1. The production process was divided into three modules:

The upstream module includes the extraction of raw materials, followed by the crushing stage in which pomace is produced, the collection of other agricultural residues useful for composting (straw, buffalo manure) and transport to the composting plant.

The core module includes the composting phase.

The downstream module includes the distribution of fertilizer to the soil.

The inventory data for the study system were gathered through a specifically designed questionnaire to capture information on all inputs utilized in the production process. These data are detailed in Table 1.

Information concerning the upstream and core modules was collected for the various composts from the following locations:

- OWC1: Motta San Giovanni farm, Motta San Giovanni Reggio Calabria, Italy;
- OWC2: Mediterranea Foods Farm, Rizziconi, Reggio Calabria, Italy;
- OWC3: Nuovo Cilento farm in San Mauro Cilento, Salerno (SA), Italy.

Regarding the downstream module, data were collected from Orfei Farm in Motta San Giovanni, Loc. Liso, Italy.

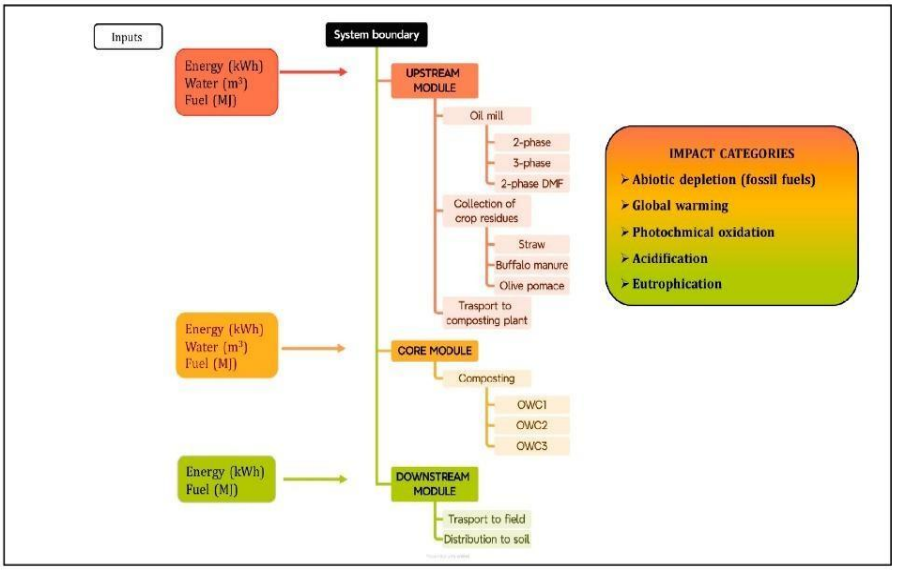


Figure 1. System boundaries divided by upstream, core, and downstream modules for assessing the environmental impacts shown in the figure of OWC1, OWC2 and OWC3.

In this study, priority was given to using primary data in terms of input material typologies and amount used. As reported by Pergola et al. [48], secondary data were additionally extrapolated from international databases of scientific importance and reliability like, Ecoinvent 3 [49] and ELCD. In particular, this was carried out for the production of diesel and electricity. For this study, impacts due to the construction of the composting facilities were not considered, only impacts due to the composting process. During the composting process, many types of gaseous compounds such as GHGs [methane (CH_4), nitrous oxide (N_2O), carbon dioxide (CO_2)] and ammonia (NH_3) are directly released into the atmosphere. The emissions of CH_4, NH_3 and N_2O were not experimentally measured, but those reported by Pergola et al. [48] were 0.24 kg of CH_4, 0.14 kg of NH_3 and 0.12 kg of N_2O per ton of feedstock. The SimaPro 9.0 software was used for assessing the environmental impacts, specifically applying the CML 2001 EU25 methodology. According to similar studies [50–54], the following impact categories were selected for this research:

- Abiotic Depletion: This category focuses on the impact of mineral and fossil fuel extraction on human health and ecosystems. The abiotic depletion factor, calculated for each extraction, reflects the decreasing availability of these non-renewable resources. This global indicator is based on reserve concentrations and depletion rates.
- Acidification: Acidifying substances negatively affect soil, groundwater, surface water, organisms, ecosystems, and materials. The impact of acidification is measured in sulfur dioxide (SO_2) equivalent per kilogram of emissions. This category is relevant on both local and global scales.
- Eutrophication: Eutrophication results from excessive levels of nitrogen dioxide (NO_2), and phosphate ions (PO_3^{4-}) and other nutrients in the environment, leading to increased production of plankton, algae, and aquatic plants. Is measured in PO_4 equivalent per kilogram of emission.
- Global Warming Potential: Greenhouse gas emissions contribute to climate change, impacting ecosystem health, human health, and material welfare. The Intergovernmental Panel on Climate Change (IPCC) developed a model to measure the global warming potential (GWP) over a 100-year period, expressed in kilograms of carbon dioxide (CO_2) equivalent per emission.
- Photochemical Oxidation: Photochemical ozone formation, a significant air pollution issue, occurs in urban areas with stagnant air and low humidity. This secondary pollutant results from complex photochemical reactions involving sunlight, nitrogen oxides (NOx), and volatile organic compounds (VOCs). Mainly produced by combustion engines and organic solvent use, photochemical ozone is also known as summer smog and is expressed in kilograms of ethylene equivalents.

2.6. Statistical Analysis

Significant differences were analyzed with Tukey multiple tests to compare all pairs of means. Simple descriptive analysis was applied to determine the average value of the quantitative variables. Normality of the data was confirmed using the Shapiro–Wilk test, justifying the use of parametric tests. Statistical analyses were performed using SYSTAT 8.0 software (SPSS Inc, Chicago, Illinois, USA). p values < 0.05 were considered significant as the probability levels. To explore the relationships among the different composts on the soil, we analyzed datasets using principal component analysis with XLStat. PCA is employed to condense large datasets by transforming variables into a smaller set of uncorrelated components, known as principal components. These components capture the maximum variance present in the original data, allowing for a simplified interpretation of complex relationships.

3. Results

3.1. Compost Characteristics

The composting procedure was repeated three times for each type of compost to ensure standardization, and the data confirmed the reproducibility of the process. After

120 days, the analysis revealed significant differences among the three composts produced using the same method (Table 2). OWC2 exhibited the most acidic pH and the highest EC value. OWC3 had the highest pH value (7.8), the highest content of TOC and TN, and the highest C/N WC. The C/N ratio exceeded 20 in all composts. The N-NH_4^+/N-NO_3^- ratio varied, being 11 in OWC2, 6.08 in OWC3, and 1.60 in OWC1. The organic nitrogen to total nitrogen (ON/TN) ratio was significantly higher in OWC1 and OWC2 (90% and 85%, respectively) compared to OWC3 (81%) (Table 2). All the composts were nutrient-rich, albeit to varying extents (Table 2). OWC2 had the highest content of nutrients, including the greatest amounts of NH_4^+ (0.28 mg/L), K^+ (20.29 mg/L), Ca^{2+} (0.56 mg/L), SO_4^2 (1.21 mg/L), and PO_4^{3-} (2.14 mg/L) (Table 3). OWC2 contained more WSP than OWC1. OWC3 was the compost with the lowest amount of WSP. OWC3 had higher CEC and DHA than OWC2 and OWC1. Conversely OWC1 had the highest FDA activity (Table 4). Phytotoxicity, an indicator of compost maturity, expressed as GI (Figure 2), showed that composts OWC1 and OWC3, tested for seed germination, were not phytotoxic. The GI, detected 6 days after germination, in presence of the composts at 50% showed values higher than 80%, falling in the class of phytonutrient. These data agree with the global germination index showing values ranging from 71 to 85% that confirmed the non-phytotoxicity of the two composts [33].

Table 2. Physical–chemical properties of composts OWC1, OWC2 and OWC3 120 days after the composting process.

ID	pH (H_2O)	pH (KCl)	EC	WC	TOC	TN	C/N	NH_4^+-N/NO_3^--N	ON/TN
OWC1	$6.44^{b*} \pm 0.11$	$5.84^{b} \pm 0.28$	$2.12^{b} \pm 0.35$	$42.6^{c} \pm 0.34$	$48.05^{b} \pm 0.28$	$1.67^{b} \pm 0.001$	$28.74^{b} \pm 0.40$	$1.60^{c} \pm 0.06$	$90.10^{a} \pm 1.68$
OWC2	$5.34^{c} \pm 0.35$	$4.88^{c} \pm 0.19$	$11.83^{a} \pm 0.80$	$58.5^{b} \pm 0.55$	$42.44^{b} \pm 0.49$	$1.64^{b} \pm 0.003$	$25.87^{b} \pm 0.51$	$11.00^{a} \pm 0.41$	$85.00^{b} \pm 1.36$
OWC3	$7.80^{a} \pm 0.17$	$7.10^{a} \pm 0.11$	$2.60^{b} \pm 0.74$	$58.3^{a} \pm 0.76$	$62.61^{a} \pm 0.56$	$1.96^{a} \pm 0.004$	$31.90^{a} \pm 0.55$	$6.08^{b} \pm 0.15$	$81.00^{c} \pm 1.45$

pH (H_2O and KCl); electric conductivity (EC, ms cm^{-1}); water content (WC,%); total organic carbon (TOC, %); total nitrogen (TN, %); carbon/nitrogen ratio (C/N); ammonium nitrogen/nitrate nitrogen ratio (NH_4^+-N/NO_3^--N); organic nitrogen/total nitrogen ratio (ON/TN, %). * Different letters in the same column indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Values are the mean of three replicates ($n = 9$) ± standard deviation.

Table 3. Cation and anion concentration (mg/L) detected in OWC1, OWC2 and OWC3 at the end of the composting process (120 days).

ID	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_2^-	NO_3^-	PO_4^{3-}	SO_4^{2-}
OWC1	$1.20^{a*} \pm 0.05$	$0.17^{b} \pm 0.01$	$17.90^{b} \pm 0.02$	$1.40^{a} \pm 0.02$	$0.29^{b} \pm 0.04$	$0.80^{b} \pm 0.11$	$0.01^{a} \pm 0.004$	$0.05^{a} \pm 0.010$	$0.50^{b} \pm 0.04$	$0.39^{b} \pm 0.03$
OWC2	$0.79^{b} \pm 0.04$	$0.28^{a} \pm 0.03$	$20.29^{a} \pm 0.01$	$1.19^{b} \pm 0.04$	$0.56^{a} \pm 0.01$	$3.77^{a} \pm 0.47$	$0.01^{a} \pm 0.003$	$0.01^{a} \pm 0.002$	$2.12^{a} \pm 0.21$	$1.21^{a} \pm 0.15$
OWC3	$1.15^{a} \pm 0.02$	$0.11^{c} \pm 0.01$	$18.43^{b} \pm 0.01$	$1.32^{a} \pm 0.12$	$0.43^{a} \pm 0.07$	$2.65^{a} \pm 0.02$	$0.02^{a} \pm 0.14$	$0.04^{a} \pm 0.002$	$1.15^{a} \pm 0.03$	$1.12^{a} \pm 0.23$

* Different letters in the same column indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Values are the mean of three replicates ($n = 9$) ± standard deviation.

Table 4. Chemical and biochemical characteristics of composts OWC1, OWC2 and OWC3 120 days after the composting process.

ID	WSPs	FDA	DHA	CEC
OWC1	$2.55^{a*} \pm 0.10$	$81.12^{a} \pm 2.16$	$39.22^{b} \pm 1.16$	$22.95^{b} \pm 0.86$
OWC2	$2.67^{a} \pm 0.11$	$16.55^{c} \pm 5.21$	$80.12^{b} \pm 3.57$	$25.13^{b} \pm 1.03$
OWC3	$1.60^{b} \pm 0.11$	$65.60^{b} \pm 2.21$	$89.31^{a} \pm 4.11$	$28.81^{a} \pm 2.81$

Water-soluble phenols (WSPs, mg TAE g^{-1} d.w.), fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.w.), dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.w.), and cation exchange capacity (CEC, cmol(+) Kg^{-1}) detected in OWC1, OWC2 and OWC3 at the end of the composting process (120 days). * Different letters in the same column indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Values are the mean of three replicates ($n = 9$) ± standard deviation.

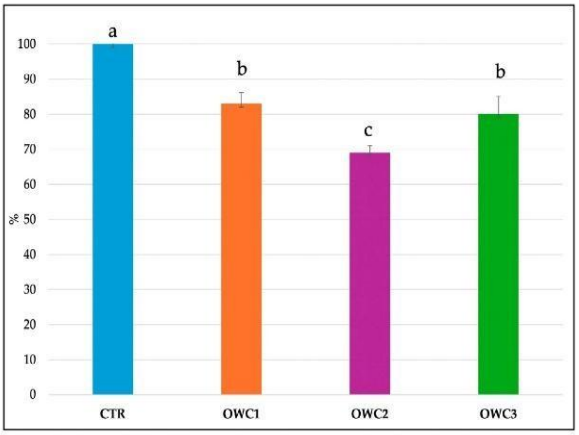


Figure 2. Global germination index of *Cucumis sativus* L. (%) in the presence of selected concentrations of the different composts. Data are the mean of 3 replications ± standard deviation. Different letters indicate significant differences (Turkey's test $p < 0.05$).

The correlation matrix of the wastes' physical–chemical properties revealed strong and positive relationships among various parameters (Figure 3). The Pearson correlation coefficient also highlighted relationships among cations and anions and between cations and anions (Figure 3). Phosphate positively correlated with all cations and anions, except for sodium, magnesium, nitrite, and nitrate; the latter two showed an inverse correlation with phosphate. Sulphate correlated positively with calcium and, to a lesser extent, with potassium, and negatively with magnesium. A positive correlation was also evident between phosphate and chloride (Figure 3).

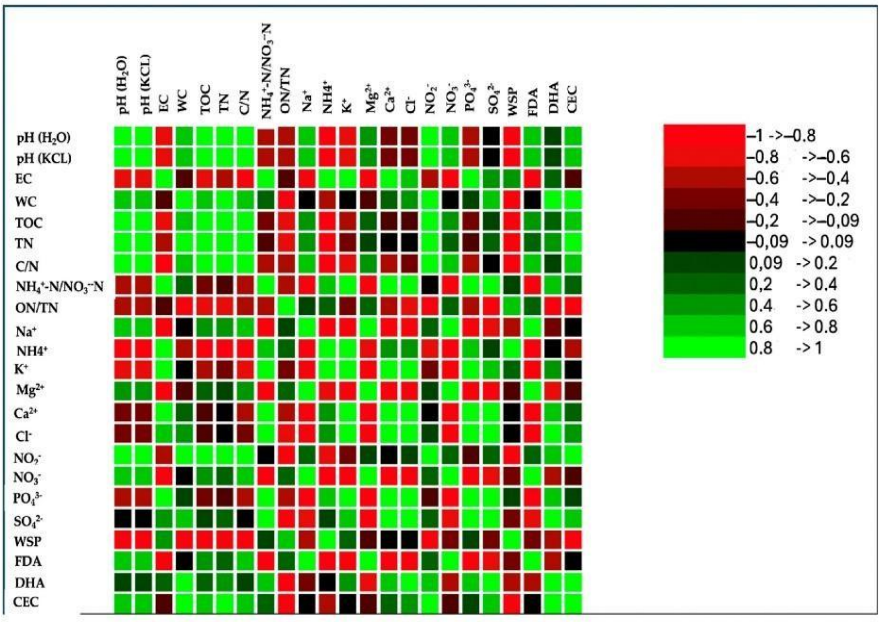


Figure 3. Correlation matrix (Pearson(n)) of chemical and biological properties and anions and cations at the end of the composting process (120 days). Green color and its shades, in the correlation matrix, indicate a positive correlation, signifying that the variables move in the same direction. On the other hand, the red color and its gradients represent an inverse correlation, suggesting that the variables move in the opposite direction.

PCA analysis showed that OWC1 is most influenced by variables pointing upwards to the left quadrant in the biplot, such as NO_3^-, ON/TN and Mg^{2+}. The position of OWC2 along the F1 axis indicates a strong correlation with NH_4^+, NH_4^+-N/NO_3^--N, Cl^-, EC, K^+, PO_4^{3-}, and SO_4^{2-}. The position of OWC3 on the lower left quadrant indicates a strong correlation with Na^+, pH and CEC (Figure 4).

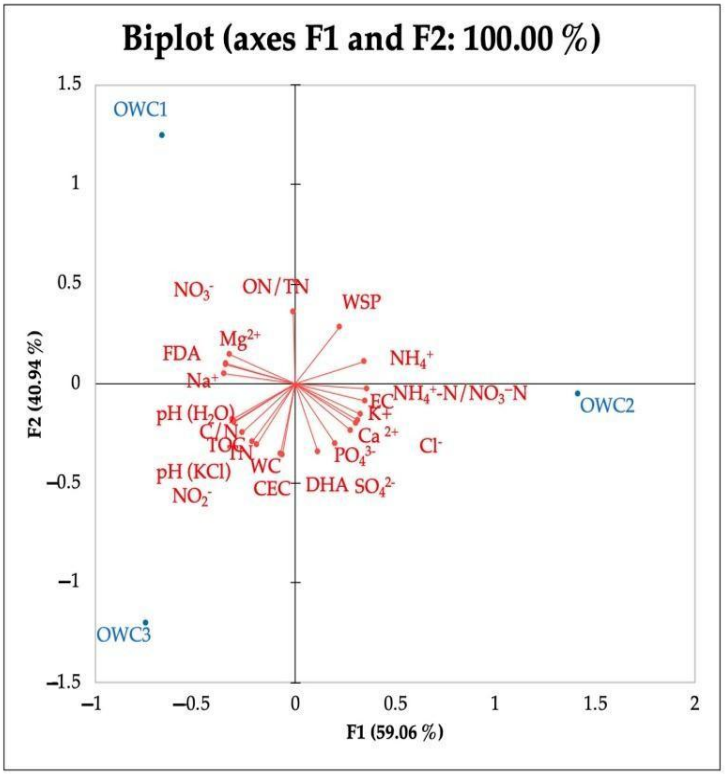


Figure 4. PCA of chemical and biological properties and anions and cations detected at the end of the composting process (120 days).

3.2. Compost Effects on Soil

All the composts affected the soil chemical properties, lowering the pH and increasing the EC in comparison to the control. The EC values in soil were, in any case, far from the threshold values of salinization (4 dS m^{-1}) (Table 5). The water-holding capacity significantly increased in treated soils as demonstrated by the values of water content. The greatest value of water content was detected in soil amended with OWC3. TOC and TN significantly increased in amended soils. The C/N ratio was the lowest in control soil, and was around 20 in all the other treatments (Table 5). WSP significantly increased in all the treatments and the greatest increase was observed in soil plus OWC2 (S+OWC2). Soil biological characteristics were affected by compost treatments to different extents. The MBC increased in the presence of the composts, and the greatest increment was observed in the presence of OWC3, followed by OWC1 and OWC2 (Table 6). OWC3 was the compost that increased the number of actinomycete, fungal, and bacterial colonies in respect to the control and the other treatments. FDA and DHA also increased with composts, much more so with OWC3 (Table 6). PCA analysis results (Figure 5) evidenced a good correlation between OWC3 treatment and biological soil properties. A significant increase in MBC and in the colony number of bacteria and actinomycetes, as well as in the activities of FDA and DHA enzymes, was observed, suggesting that from a microbial point of view, compost application is an environmentally friendly and rapid measure for restoring soil functionality.

No significant correlations between soil amended with OWC2 and biological soil properties were detected. Soil with OWC1 correlated with fungi, TOC and WSP. Correlation matrix results (Figure 6) evidenced a significant correlation among water content, TOC, TN, C/N, MBC, fungi, bacteria, and actinomycetes, as well as FDA and DHA.

Cation and anion amounts changed after the addition of the composts. All the composts increased the amount of K^+. OWC1 and OWC2 increased the amount of Ca^{2+} and Mg^{2+} in soil. PO_4^{3-} increased in soil treated with OWC3, and SO_4^{2-} only when OWC1 and OWC3 were used. OWC2 did not change the amount of SO_4^{2-} in soil respect to the control. CEC was higher than the control in soil treated with all the composts (Table 7). These data are supported by PCA data analysis (Figure 5).

Table 5. Soil chemical characteristics detected 6 months after the addition of compost to the soils. Soil plus compost 1 (S+OWC1), soil plus compost 2 (S+OWC2), soil plus compost 3 (S+OWC3), and unamended soil (control, CTR).

ID	pH	EC	WC	TOC	TN	C/N	WSPs
CTR	8.50 ^{a*} ± 0.04	338 ^{c} ± 7.2	20.7 ^{c} ± 1.1	3.0 ^{b} ± 0.05	0.18 ^{b} ± 0.003	16.6 ^{b} ± 0.06	15 ^{c} ± 1.120
S+OWC1	8.00 ^{b} ± 0.05	455 ^{a} ± 5.7	29.2 ^{a} ± 0.9	4.1 ^{b} ± 0.23	0.21 ^{a} ± 0.002	20.0 ^{a} ± 0.01	43 ^{b} ± 1.073
S+OWC2	8.00 ^{b} ± 0.08	398 ^{b} ± 7.1	23.9 ^{b} ± 1.6	4.2 ^{b} ± 0.21	0.20 ^{ab} ± 0.001	21.0 ^{a} ± 0.03	51 ^{a} ± 1.601
S+OWC3	8.01 ^{b} ± 0.01	400 ^{b} ± 3.1	35.2 ^{c} ± 0. 8	4.9 ^{a} ± 0.12	0.25 ^{a} ± 0.004	19.6 ^{a} ± 0.08	44 ^{b} ± 2.08

pH (H_2O), electric conductivity (EC, µS cm^{-1}), water content (WC, %), total organic carbon (TOC, %), total nitrogen (TN, %), carbon/nitrogen ratio (C/N), water-soluble phenols (WSPs, µg TAE g^{-1} d.s.). * Different letters in the same column indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Values are the mean of three replicates ($n = 12$) ± standard deviation.

Table 6. Biological properties of soil plus compost 1 (S+OWC1), soil plus compost 2 (S+OWC2), soil plus compost 3 (S+OWC3), and unamended soil (control, CTR).

ID	MBC	FDA	DHA	BACT	FUN	ACTINOM
CTR	401 ^{d*} ± 8.7	2.9 ^{c} ± 0.05	19.8 ^{c} ± 0.11	$1.3 \times 10^{5\,c}$ ± 0.21	$4.4 \times 10^{4\,c}$ ± 0.53	$6.1 \times 10^{4\,c}$ ± 0.47
S+OWC1	550 ^{b} ± 4.5	3.5 ^{b} ± 0.02	24.6 ^{b} ± 0.02	$1.4 \times 10^{5\,c}$ ± 0.19	$1.2 \times 10^{5\,b}$ ± 1.28	$1.5 \times 10^{5\,b}$ ± 0.22
S+OWC2	466 ^{c} ± 4.9	3.1 ^{c} ± 0.01	20.20 ^{c} ± 0.06	$2.0 \times 10^{5\,b}$ ± 0.13	$1.1 \times 10^{5\,b}$ ± 0.89	$1.2 \times 10^{5\,b}$ ± 0.27
S+OWC3	580 ^{a} ± 8.3	4.1 ^{a} ± 0.05	28.10 ^{a} ± 0.32	$2.4 \times 10^{5\,a}$ ± 0.11	$1.5 \times 10^{5\,a}$ ± 0.53	$2.0 \times 10^{5\,a}$ ± 0.13

Microbial biomass C (MBC, µg C g^{-1} f.s.), fluorescein di-acetate (FDA, µg fluorescein g^{-1} d.s.), dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.s.), bacteria (BACT, UFC g^{-1} d.s.), fungi (FUN, UFC g^{-1} d.s.), actinomycetes (ACTINOM, UFC g^{-1} d.s.). * Different letters in the same column indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Values are the mean of three replicates ($n = 12$) ± standard deviation.

Table 7. Cations (mg g^{-1} d.s.), anions (mg g^{-1} d.s.) and cation exchange capacity (CEC, $cmol^{(+)}$ Kg^{-1}) detected 6 months after the addition of compost to the soils. Soil plus compost 1 (S+OWC1), soil plus compost 2 (S+OWC2), soil plus compost 3 (S+OWC3), and unamended soil (control, CTR).

ID	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	CEC
CTR	0.12 ^{c*} ± 0.001	0.11 ^{c} ± 0.004	0.023 ^{b} ± 0.001	0.86 ^{a} ± 0.050	0.22 ^{b} ± 0.007	0.022 ± 0.0004	0.001 ^{b} ± 0.002	0.34 ^{c} ± 0.050	18.7 ^{b} ± 0.101
S+OWC1	0.15 ^{b} ± 0.005	0.15 ^{b} ± 0.002	0.029 ^{a} ± 0.002	0.47 ^{c} ± 0.011	0.27 ^{a} ± 0.020	nd	nd	0.68 ^{a} ± 0.101	22.3 ^{a} ± 0.310
S+OWC2	0.12 ^{c} ± 0.001	0.18 ^{a} ± 0.001	0.029 ^{a} ± 0.001	0.50 ^{c} ± 0.010	0.18 ^{b} ± 0.011	nd	nd	0.34 ^{c} ± 0.073	21.8 ^{a} ± 0.105
S+OWC3	0.18 ^{a} ± 0.002	0.21 ^{a} ± 0.003	0.019 ^{b} ± 0.002	0.57 ^{b} ± 0.001	0.17 ^{b} ± 0.031	nd	0.005 ^{a} ± 0.011	0.58 ^{b} ± 0.001	21.3 ^{a} ± 0.001

Different letters in the same column indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Values are the mean of three replicates ($n = 12$) ± standard deviation.

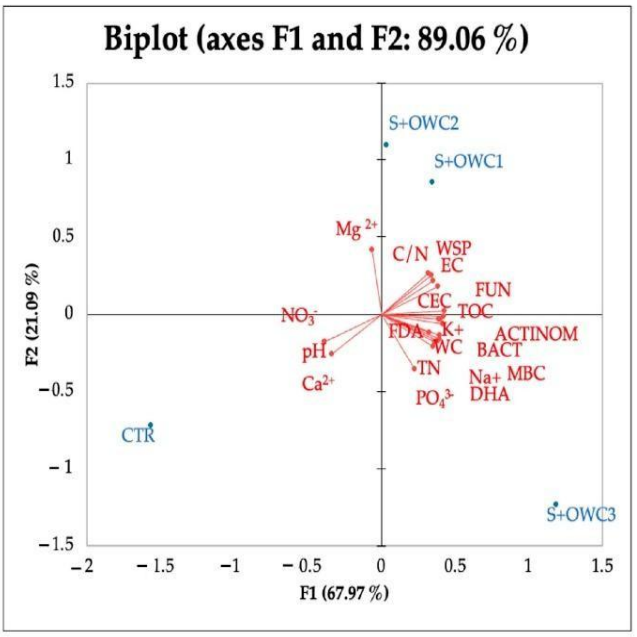


Figure 5. PCA of chemical and biological properties and anions and cations detected in soil amended with compost 1 (S+OWC1), compost 2 (S+OWC2), compost 3 (S+OWC3), and unamended soil (control, CTR). pH, electric conductivity (EC, dS m^{-1}), water content (WC, %), total organic carbon (TOC, %), total nitrogen (TN, %), carbon/nitrogen ratio (C/N), water-soluble phenols (WSPs, µg TAE g^{-1} d.s.), microbial biomass C (MBC, µg C g^{-1} f.s.), fluorescein di-acetate (FDA, µg fluorescein g^{-1} d.s.), dehydrogenase (DHA, µg TTF g^{-1} h^{-1} d.s.), bacteria (BACT, UFC g^{-1} d.s.), fungi (FUN, UFC g^{-1} d.s.), and actinomycetes (ACTINOM, UFC g^{-1} d.s.).

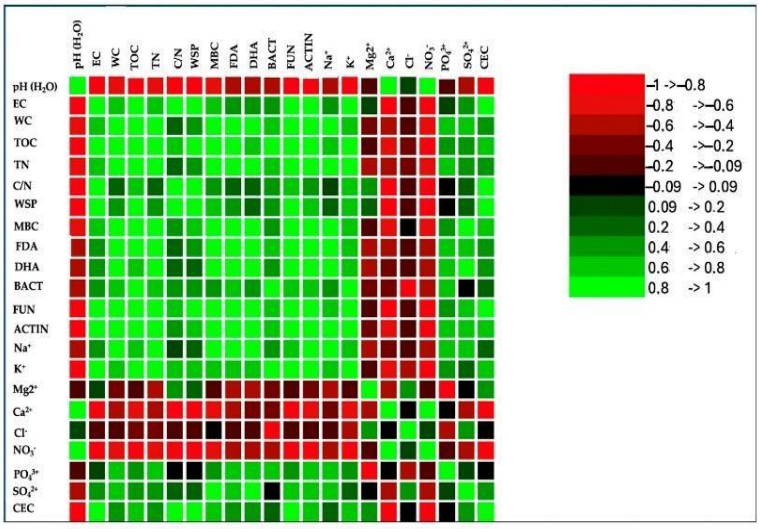
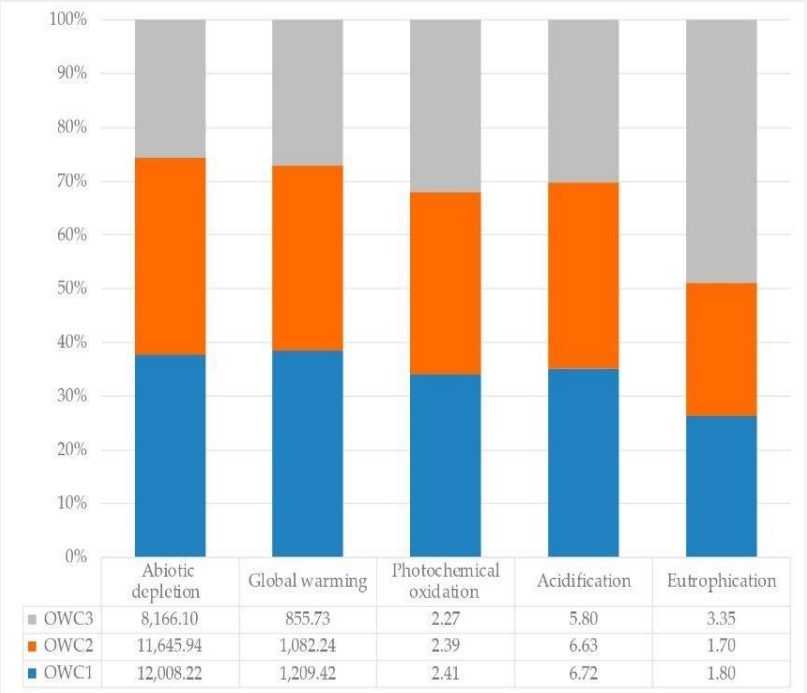


Figure 6. Correlation matrix of chemical and biological properties of soil plus compost 1 (S+OWC1), compost 2 (S+OWC2), compost 3 (S+OWC3), and unamended soil (control, CTR). Values in bold are different from 0 with a significance level alpha = 0.05. Green color and its shades in the correlation matrix indicate a positive correlation, signifying that the variables move the same direction. On the other hand, the red color and its gradients represent an inverse correlation, suggesting that the variables move in opposite directions.

3.3. Environmental Impact

The environmental impact results, based on the functional unit (1 ton of compost), are summarized in Figure 7. OWC1 had a greater impact than the other two composts on all categories (abiotic depletion (fossil fuels), photochemical oxidation, acidification, global warming potential), except for the eutrophication impact category, where the most impactful compost was OWC3.



	Abiotic depletion	Global warming	Photochemical oxidation	Acidification	Eutrophication
OWC3	8,166.10	855.73	2.27	5.80	3.35
OWC2	11,645.94	1,082.24	2.39	6.63	1.70
OWC1	12,008.22	1,209.42	2.41	6.72	1.80

Figure 7. Environmental impact per ton of OWC1, OWC2 and OWC3 on individual impact categories: eutrophication (kg PO_4 eq), acidification (kg SO_2 eq), photochemical oxidation (kg C_2H_4 eq), abiotic depletion (fossil fuels) (MJ), and global warming (kg CO_2 eq).

From decomposition to the cradle-to-gate production processes of the three composts under study (Table 8), it was found that for all the impact categories, the most impactful processes were the upstream ones, i.e., the production process step concerning raw material procurement, including the oil mill, the collection of crop residues (olive pomace, straw and manure), and transport to the composting plant. Specifically, for the impact categories, the ranking of composts for the upstream module was abiotic depletion (fossil fuels) (OWC1 > OWC2 > OWC3), global warming (OWC1 > OWC2 > OWC3), photochemical oxidation (OWC1 = OWC2 > OWC3), acidification (OWC1 > OWC2 > OWC3), and eutrophication (OWC3 > OWC1 > OWC2). For the core processes, which refer to the composting process itself, the ranking of composts was abiotic depletion (fossil fuels) (OWC1 > OWC2 > OWC3), global warming (OWC1 = OWC2 > OWC3), photochemical oxidation (OWC1 > OWC2 > OWC3), acidification (OWC1 > OWC2 > OWC3) and eutrophication (OWC1 = OWC2 = OWC3). For the downstream processes, which encompass the transportation and application of the compost, the ranking of composts was abiotic depletion (fossil fuels) (OWC2 > OWC1 > OWC3), global warming (OWC1 = OWC2 > OWC3), photochemical oxidation (OWC1 > OWC2 > OWC3), acidification (OWC1 = OWC2 > OWC3), and eutrophication (OWC1 = OWC2 > OWC3).

Table 8. Environmental impact per ton of compost OWC1, OWC2 and OWC3 in the entire life cycle assessment.

Impact Categories	OWC1			OWC2			OWC3		
	Upstream	Core	Downstream	Upstream	Core	Downstream	Upstream	Core	Downstream
Abiotic depletion	10,763.7	295	949.5	10,401.7	294.7	959.5	7397.3	187.7	581
Global warming	1005.8	19.3	84.4	978.6	19.3	84.4	790.7	14.4	50.6
Photochemical oxidation	0.4	0	0	0.4	0	0	0.3	0	0
Acidification	6.2	0.1	0.4	6.1	0.1	0.4	5.5	0	0.3
Eutrophication	1.6	0	0.2	1.5	0	0.2	3.2	0	0.1

Abiotic depletion (fossil fuels) (MJ), global warming (kg CO_2 eq), photochemical oxidation (kg C_2H_4 eq), acidification (kg SO_2 eq) and eutrophication (kg PO_4 eq).

4. Discussion

Applying compost to agricultural land is widely recognized as an effective method of enhancing the physical properties of most soils, especially those with poor structure and low organic matter content [55]. There is growing interest in using compost to rehabilitate soils and improve their functionality [56]. Documented improvements in compost-amended soils evidenced changes in bulk density, infiltration rate, hydraulic conductivity, water content, aggregate stability, and porosity [57]. These beneficial effects are interactive and are largely due to the compost materials used and the organic matter content in the compost feedstock.

The addition of compost to the soil initiates the decomposition of organic materials by numerous soil organisms [58], collectively known as the soil food web, with a consequent increase in soil biodiversity. These organisms with their diversity are able to recycle the organic materials back into the soil, maintaining its quality and functionality. Decomposition generates a diverse array of carbon-based compounds, including simple sugars that drive biological activity, cellulose-binding agents that enhance soil structure, and nutrient release [59]. There is a cyclical process that begins with the feedstock used to produce compost, which in turn determines its composition and its effectiveness in improving soil health and fertility, and concludes with increases in productivity. Our results confirmed the importance of feedstock composition in producing compost. Even though the feedstocks used came entirely from the olive industry, their composition varied depending on the oil production system. In fact, despite using the same composting processes, the resulting composts differed in their chemical features, underscoring the significance of the chemical composition of raw materials. Considered together, the indicators examined the quality ranking of the obtained composts in terms of their effectiveness on soil, which was in the order OWC3 > OWC2 > OWC1. All the composts decreased soil pH due to their intrinsic pH, and increased EC due to the addition of salts to soil, in particular anions, and due to the stimulation of soil microorganisms that are involved in the turnover of organic matter and the release of single nutrients. Additionally, an increase in soil organic carbon, CEC, and the C/N ratio was also observed with all the composts. These results evidenced the generation of a positive feedback loop for carbon storage by maintaining a high dissolved organic C content, increasing microbial biomass and nutrients. These processes, in turn, can promote the ability of the microbial community to utilize more diverse C sources that are already present in or added to soil. These results were further supported by the increased presence of fungi, actinomycetes, and bacteria, the principal organisms involved in the transformation and decomposition of organic matter and in the mineralization process. PCA results supported these findings, evidencing a strict correlation between OWC3 and biological parameters (MBC, bacteria, actinomycetes, DHA and FDA) (Figure 5). It is well known that microbial changes accompany changes in nutrient status to drive SOM decomposition. Fungi, bacteria and actinomycetes are crucial for organic matter decomposition and nitrogen mineralization in soil, each with distinct decomposition rates and efficiencies, playing varying roles across diverse soil ecosystems [60]. In bacteria-dominated soils, bacteria accelerate organic matter decomposition and nutrient mineralization, thereby

enhancing nutrient availability. Conversely, in fungus-dominated soils, fungi slow down the conversion rate of nutrients and energy, promoting organic matter storage and nutrient retention [61–63]. Our results showed a greater increase in microbial biomass, bacteria, fungi, actinomycetes, and enzymes in soils amended with OWC3. These results suggest intense biological activity, driving the release of nutrients that are utilizable by plants, and also evidence carbon storage due to the greatest number of fungi as well as a C/N ratio value of 20, which indicate an equilibrium state between mineralization and immobilization processes [63]. In this study, we highlighted that all the composts significantly improved soil stability, water-holding capacity, soil fertility and microbial community in respect to the control, although to different extents, and among the composts, OWC3 was the compost with the best effectiveness in soil. This highlighted the importance of the chemical composition of raw materials more than the setup parameters of composting processes in determining the stability and quality of composts.

Regarding environmental impact assessment, OWC1 and OWC2 had higher values of abiotic depletion (MJ) and global warming (Kg CO_2 eq) than OWC3, mainly due to the high consumption of electricity and diesel in the crushing, harvesting, and composting stages. OWC3, despite using more resources such as straw and buffalo manure, had a lower impact due to lower overall energy consumption.

Emissions of VOCs and acid gasses (SO_2 and NOx) were higher in OWC1 and OWC2, adversely affecting photochemical oxidation (kg C_2H_4 eq) and acidification (kg SO_2 eq), which are related to higher diesel use. OWC3 had a significantly greater impact on eutrophication (kg PO_4 eq) due to the high nutrient content in the buffalo manure used. Reductions in the electricity and diesel consumption at all stages can have multiple benefits on different categories of environmental impact. Optimizing nutrient management and transport logistics is crucial to minimize eutrophication and other harmful emissions.

In summary, improving energy efficiency and resource management during the various stages of the compost life cycle can significantly reduce overall environmental impacts.

Furthermore, when OWC1, OWC2, and OWC3 composts were applied to the soil, they exhibited reduced environmental impacts compared to the impacts reported by other studies for NPK synthetic fertilizers—in which the global warming is 2107 kg CO_2 eq per ton, the acidification is 2001 Kg of SO_2 eq per ton, and the eutrophication is 2.93 kg of PO_4 eq per ton [64]—and compost derived from food wastes [65], in which the global warming is 6190 kg of CO_2 eq per ton and acidification is 4820 Kg of SO_2 eq per ton. Furthermore, among the composts used, OWC3 showed a lower impact on the soil than the other two, possibly due to its lower phenol content and higher C/N ratio. The lower phenol content may stem from the fact that the compost was prepared using a lower percentage of olive waste, which was replaced by manure. The higher C/N ratio, also a result of the initial material percentages, suggests a higher stable fraction of organic matter, which is reflected in the slow release of nutrients.

The production of compost from recalcitrant wastes of the olive food processing industry can be, in any case, an opportunity for the soil, the environment, and the economy, with a significant improvement in soil fertility and a great reduction in greenhouse gas emissions [66] and waste disposal costs [67,68].

5. Conclusions

In conclusion, this study underscores the substantial advantages of repurposing olive oil extraction wastes into compost for sustainable agricultural practices. This research demonstrates that both the proportion of olive oil waste and the olive oil extraction methods significantly impact the compost quality and its environmental footprint. Incorporating organic waste into soil not only enhances soil's chemical and biochemical attributes by enriching it with essential nutrients but also bolsters its long-term sustainability. Furthermore, this approach presents an effective strategy for olive waste management, offering notable environmental benefits and positively influencing the olive oil supply chain's economy. Moving forward, embracing composting techniques could transform waste management

within the olive industry, fostering a more robust and environmentally conscious agricultural sector. Agriculture is the biggest market for compost; trials have shown that quality compost can significantly improve the long-term health of the soil. But it can also benefit the farming economy.

Future investigations could delve into novel waste material combinations and refine composting methodologies to amplify soil advantages while mitigating environmental repercussions. Such advancements will not only fortify sector sustainability but also advance a circular economy paradigm, where waste evolves into valuable resources for agricultural cultivation.

Author Contributions: Conceptualization, A.M. (Adele Muscolo) and G.C.; writing—original draft preparation, A.M. (Adele Muscolo) and A.M. (Angela Maffia); writing—review and editing, A.M. (Angela Maffia), M.I.H. and A.M. (Adele Muscolo); supervision, A.M. (Adele Muscolo) and G.C.; project administration, A.M. (Adele Muscolo); software, A.M. (Angela Maffia) and F.M.; formal analysis, C.M. and M.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Italian Ministry for University and Research (MUR), Project CN_00000022, "National Research Centre for Agricultural Technologies-Agritech" and "Solutions for soil quality assessment and protection" 3.2.1.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Farnia, A.; Hassanpour, K. Comparison between effect of chemical and biological fertilizers on yield and yield components of wheat (*Triticum aestivum* L.) Pishtaz cultivar. *Indian J. Sci.* **2015**, *5*, 7792–7808.
2. Mariyam, S.; Upadhyay, S.K.; Chakraborty, K.; Verma, K.K.; Duhan, J.S.; Muneer, S.; Meena, M.; Sharma, R.K.; Ghodake, G.; Seth, C.S. Nanotechnology, a frontier in agricultural science, a novel approach in abiotic stress management and convergence with new age medicine-A review. *Sci. Total Environ.* **2024**, *912*, 169097. [CrossRef]
3. Ferreira, C.S.S.; Seifollahi-Aghmiuni, S.; Destouni, G.; Ghajarnia, N.; Kalantari, Z. Soil degradation in the European Mediterranean region: Processes, status and consequences. *Sci. Total Environ.* **2022**, *805*, 150106. [CrossRef] [PubMed]
4. Haddaway, N.R.; Hedlund, K.; Jackson, L.E.; Kätterer, T.; Lugato, E.; Thomsen, I.K.; Jørgensen, H.B.; Söderström, B. What are the effects of agricultural management on soil organic carbon in boreo-temperate systems? *Environ. Evid.* **2015**, *4*, 23. [CrossRef]
5. Bisht, N.; Chauhan, P.S. Excessive and disproportionate use of chemicals cause soil contamination and nutritional stress. In *IntechOpen eBooks*; IntechOpen: London, UK, 2021. [CrossRef]
6. Alessandri, A.; De Felice, M.; Zeng, N.; Mariotti, A.; Pan, Y.; Cherchi, A.; Lee, J.-Y.; Wang, B.; Ha, K.-J.; Ruti, P.; et al. Robust assessment of the expansion and retreat of Mediterranean climate in the 21st century. *Sci. Rep.* **2014**, *4*, 7211. [CrossRef] [PubMed]
7. Calicioglu, O.; Flammini, A.; Bracco, S.; Bellù, L.; Sims, R. The Future Challenges of Food and Agriculture: An Integrated analysis of Trends and solutions. *Sustainability* **2019**, *11*, 222. [CrossRef]
8. Upadhyay, S.K.; Singh, G.; Rani, N.; Rajput, V.D.; Seth, C.S.; Dwivedi, P.; Minkina, T.; Wong, M.H.; Show, P.L.; Khoo, K.S. Transforming bio-waste into value-added products mediated microbes for enhancing soil health and crop production: Perspective views on circular economy. *Environ. Technol. Innov.* **2024**, *34*, 103573. [CrossRef]
9. Istat, Censimento Agricoltura 2020. Available online: https://esploradati.istat.it/databrowser/#/it/censimentoagricoltura (accessed on 10 April 2024).
10. Donner, M.; Radić, I. Innovative circular business models in the olive oil sector for sustainable Mediterranean agrifood systems. *Sustainability* **2021**, *13*, 2588. [CrossRef]
11. Enaime, G.; Dababat, S.; Wichern, M.; Lübken, M. Olive mill wastes: From wastes to resources. *Environ. Sci. Pollut. Res. Int.* **2024**, *31*, 20853–20880. [CrossRef]
12. Markou, G.; Georgakakis, D.; Plagou, K.; Salakou, G.; Christopoulou, N. Balanced waste management of 2-and 3-phase olive oil mills in relation to the seed oil extraction plant. *Terr. Aquat. Environ. Toxicol.* **2010**, *4*, 109–112.
13. Directive, E.C. Directive 2008/98/EC of The European Parliament and of The Council on Waste and Repealing Certain Directives. *Off. J. Eur. Union* **2008**, *312*, 3–30.
14. Brunetti, G.; Plaza, C.; Senesi, N. Olive pomace amendment in Mediterranean conditions: Effect on soil and humic acid properties and wheat (*Triticum turgidum* L.) yield. *J. Agric. Food Chem.* **2005**, *53*, 6730–6736. [CrossRef] [PubMed]
15. Roig, A.; Cayuela, M.L.; Sànchez-Monedero, M.A. An overview on olive mill wastes and their valorization methods. *Waste Manag.* **2006**, *26*, 960–969. [CrossRef] [PubMed]
16. Alburquerque, J.A.; Gonzálvez, J.; García, D.; Cegarra, J. Composting of a solid olive-mill by-product ("alperujo") and the potential of the resulting compost for cultivating pepper under commercial conditions. *Waste Manag.* **2006**, *26*, 620–626. [CrossRef] [PubMed]

17. Muscolo, A.; Papalia, T.; Settineri, G.; Mallamaci, C.; Jeske-Kaczanowska, A. Are raw materials or composting conditions and time that most influence the maturity and/or quality of composts? Comparison of obtained composts on soil properties. *J. Clean. Prod.* **2018**, *195*, 93–101. [CrossRef]
18. Vignozzi, N.; Andrenelli, M.C.; Agnelli, A.E.; Fiore, A.; Pellegrini, S. Short-Term Effect of Different Inputs of Organic Amendments from Olive Oil Industry By-Products on Soil Organic Carbon and Physical Properties. *Land* **2023**, *12*, 1628. [CrossRef]
19. Cayuela, M.L.; Bernal, M.P.; Roig, A. Composting olive mill waste and sheep manure for orchard use. *Compost. Sci. Util.* **2004**, *12*, 130–136. [CrossRef]
20. Garcia-Gomez, A. Growth of ornamental plants in two composts prepared from agroindustrial wastes. *Bioresour. Technol.* **2002**, *83*, 81–87. [CrossRef] [PubMed]
21. Alfano, G.; Lustrato, G.; Lima, G.; Vitullo, D.; Ranalli, G. Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. *Biol. Control* **2011**, *58*, 199–207. [CrossRef]
22. López-Piñeiro, A.A.; Albarrán, J.M.; Rato Nunes, C. Barreto, Short and medium-term effects of two-phase olive mill waste application on olive grove production and soil properties under semiarid mediterranean conditions. *Bioresour. Technol.* **2008**, *99*, 7982–7987; ISSN 0960-8524. [CrossRef]
23. Liang, C.; Das, K.C.; McClendon, R.W. The Influence of Temperature and Moisture Contents Regimes on the Aerobic Microbial Activity of a Biosolids Composting Blend. *Bioresour. Technol.* **2003**, *86*, 131–137. [CrossRef] [PubMed]
24. ANPA (National Agency for Environmental Protection). *National Agency for Environmental Protection Guidelines. "Methods of Compost Analysis", Manuals and Guidelines 3/2001*; 6334 Manuali 3; SPED S.r.l.: Rome, Italy, 2001; ISBN 88-448-0258-9.
25. Adam, G.; Duncan, H. Development of a sensitive and rapid method for the measurement of total microbial activity usingfluorescein diacetate (FDA) in a range of soils. *Soil Biol. Biochem.* **2001**, *33*, 943–951. [CrossRef]
26. Perucci, P. Enzyme activity and microbial biomass in a field soil amended with municipal 460 refuse. *Biol. Fertil. Soils* **1992**, *14*, 54–60. [CrossRef]
27. Von Mersi, W.; Schinner, F. An improved and accurate method for determining the dehydrogenase activity of soils withiodonitrotetrazolium chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. [CrossRef]
28. Box, J.D. Investigation of the Folin-Ciocalteau phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* **1983**, *17*, 511–525. [CrossRef]
29. Muscolo, A.; Papalia, T.; Settineri, G.; Romeo, F.; Mallamaci, C. Three different methods for turning olive pomace in resource: Benefits of the end products for agricultural purpose. *Sci. Total Environ.* **2019**, *662*, 1–7. [CrossRef] [PubMed]
30. Mehlich, A. Rapid Determination of Cation and Anion Exchange Properties and pHe of Soils. *J. Assoc. Off. Agric. Chem.* **1953**, *36*, 445–457. [CrossRef]
31. Gariglio, N.; Buyatti, M.; Pilatti, R.A.; Russia, D.E.G.; Acosta, M.R. Use of a germination bioassay to test compost maturity ofwillow (*Salix* sp.) sawdust. *N. Z. J. Crop Hortic. Sci.* **2002**, *30*, 135–139. [CrossRef]
32. Tiquia, S.M.; Tam, N.F.Y. Elimination of phytotoxicity during co-composting of spent pig-manure sawdust litter and pig sludge. *Bioresour. Technol.* **1998**, *65*, 43–49. [CrossRef]
33. Zucconi, F.; Pera, A.; Forte, M.; De Bertoldi, M. Evaluating toxicity of immature compost. *BioCycle* **1981**, *22*, 54–57.
34. Association of Official Analytical Chemist. *Official Methods of Analysis*, 15th ed.; Association of Official Analytical Chemist: Washington, DC, USA, 1990.
35. Da Silva, E.F.; Melo, M.F.; Sombra, K.E.S.; Silva, T.S.; De Freitas, D.F.; Da Costa, M.E.; Da Silva Santos, E.P.; Da Silva, L.F.; Serra, A.P.; De Morais Cavalcante Neitzke, P.R. Organic nitrogen in agricultural systems. In *IntechOpen eBooks*; IntechOpen: London, UK, 2020. [CrossRef]
36. FAO. *Methods of Analysis for Soils of Arid and Semi-Arid Regions*; Food and Agricultural Organization: Rome, Italy, 2007; p. 57.
37. Klute, A. *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods*, 2nd ed.; Agronomy Monograph 9; ASA-SSSA: Madison, WI, USA, 1986.
38. Bouyoucos, G.J. Hydrometer method improved for making particle size analysis of soils. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
39. Muscolo, A.; Settineri, G.; Papalia, T.; Attinà, E.; Basile, C.; Panuccio, M.R. (Anaerobic co-digestion of recalcitrant agricultural wastes: Characterizing of biochemical parameters of digestate and its impacts on soil ecosystem. *Sci. Total Environ.* **2017**, *586*, 746–752. [CrossRef] [PubMed]
40. Walkley, A.; Black, I.A. An examination of the Degtjareff method for determining soil organic matter and a proposed modificationof the chromic acid titration method. *Soil Sci.* **1934**, *37*, 29–38. [CrossRef]
41. Kjeldahl, J. Neue Methode zur Bestimmung des Stickstoff in organishen Kopern. *Anal. Chem.* **1883**, *22*, 354–358.
42. Vance, E.D.; Brookes, P.C.; Jenkinson, D.S. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* **1987**, *19*, 703–707. [CrossRef]
43. Insam, H.; Goberna, M. *Section 4 Update: Use of Biolog® for the Community Level Physiological Profiling (CLPP) of Environmental Samples*; Springer eBooks: Berlin/Heidelberg, Germany, 2008; pp. 2755–2762.
44. Waksman, S.A. *Soil Microbiology*; John Wiley & Sons: New York, NY, USA, 1952.
45. Johnson, L.F.; Curl, E.A. *Methods for the Research on Ecology of Soil-Borne Plant Pathogens*; Burgess Publishing Co.: Minneapolis, MN, USA, 1972.
46. *UNI EN ISO 14040:2006*; Environmental Management—Life Cycle Assessment—Principles and Framework. International Organization for Standardization: Geneva, Switzerland, 2006.

47. *UNI EN ISO 14044:2006*; Environmental Management, Life Cycle Assessment—Requirements and Guidelines. International Organization for Standardization (ISO): Geneva, Switzerland, 2006.
48. Pergola, M.; Persiani, A.; Pastore, V.; Palese, A.M.; D'Adamo, C.; De Falco, E.; Celano, G. Sustainability Assessment of the Green Compost Production Chain from Agricultural Waste: A Case Study in Southern Italy. *Agronomy* **2020**, *10*, 230. [CrossRef]
49. Ecoinvent Version 3. 2013. Available online: http://www.ecoinvent.org/database/database.html (accessed on 4 April 2024).
50. Cadena, E.; Colón, J.; Artola, A.; Sánchez, A.; Font, X. Environmental impact of two aerobic composting technologies using life cycle assessment. *Int. J. Life Cycle Assess.* **2009**, *14*, 401–410. [CrossRef]
51. Banar, M.; Cokaygil, Z.; Ozkan, A. Life cycle assessment of solid waste management options for Eskisehir, Turkey. *Waste Manag.* **2009**, *29*, 54–62. [CrossRef]
52. Blengini, G.A. Using LCA to evaluate impacts and resources conservation potential of composting: A case study of the Asti District in Italy. *Resour. Conserv. Recycl.* **2008**, *52*, 1373–1381. [CrossRef]
53. Emery, A.; Davies, A.; Griffiths, A.; Williams, K. Environmental and economic modelling: A case study of municipal solid waste management in Wales. *Resour. Conserv. Recycl.* **2007**, *49*, 244–263. [CrossRef]
54. Eriksson, O.; Reich, M.; Frostell, B.; Björklund, A.; Assefa, G.; Sundquvist, J.; Granath, J.; Baky, A.; Thyselius, L. Municipal solid waste management from a systems perspective. *J. Clean. Prod.* **2005**, *13*, 241–252. [CrossRef]
55. Matisic, M.; Dugan, I.; Bogunovic, I. Challenges in Sustainable Agriculture—The Role of Organic Amendments. *Agriculture* **2024**, *14*, 643. [CrossRef]
56. Scotti, R.; Bonanomi, G.; Scelza, R.; Zoina, A.; Rao, M. Organic amendments as sustainable tool to recovery fertility in intensive agricultural systems. *J. Soil Sci. Plant Nutr.* **2015**, *15*, 333–352. [CrossRef]
57. Kranz, C.N.; McLaughlin, R.A.; Johnson, A.; Miller, G.; Heitman, J.L. The effects of compost incorporation on soil physical properties in urban soils—A concise review. *J. Environ Manag.* **2020**, *261*, 110209. [CrossRef] [PubMed]
58. Lin, C.; Cheruiyot, N.K.; Bui, X.-T.; Ngo, H.H. Composting and its application in bioremediation of organic contaminants. *Bioengineered* **2022**, *13*, 1073–1089. [CrossRef] [PubMed]
59. Hoffland, E.; Kuyper, T.W.; Comans, R.N.J.; Creamer, R.E. Eco-functionality of organic matter in soils. *Plant Soil* **2020**, *455*, 1–22. [CrossRef]
60. Maron, P.-A.; Sarr, A.; Kaisermann, A.; Lévêque, J.; Mathieu, O.; Guigue, J.; Karimi, B.; Bernard, L.; Dequiedt, S.; Terrat, S.; et al. High microbial diversity promotes soil ecosystem functioning. *Appl. Environ. Microbiol.* **2018**, *84*, e02738-17. [CrossRef] [PubMed]
61. Six, J.; Frey, S.D.; Thiet, R.K.; Batten, K.M. Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci. Soc. Am. J.* **2006**, *70*, 555–569. [CrossRef]
62. Strickland, M.S.; Rousk, J. Considering fungal:bacterial dominance in soils—Methods, controls, and ecosystem implications. *Soil Biol. Biochem.* **2010**, *42*, 1385–1395. [CrossRef]
63. Waring, B.G.; Averill, C.; Hawkes, C.V. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: Insights from meta-analysis and theoretical models. *Ecol. Lett.* **2013**, *16*, 887–894. [CrossRef]
64. El Chami, D.; Santagata, R.; Moretti, S.; Moreschi, L.; Del Borghi, A.; Gallo, M. A Life Cycle Assessment to Evaluate the Environmental Benefits of Applying the Circular Economy Model to the Fertiliser Sector. *Sustainability* **2023**, *15*, 15468. [CrossRef]
65. Saer, A.; Lansing, S.; Davitt, N.H.; Graves, R.E. Life cycle assessment of a food waste composting system: Environmental impact hotspot. *J. Clean. Prod.* **2012**, *52*, 234–244. [CrossRef]
66. Bong, C.P.-C.; Goh, R.K.Y.; Lim, J.-S.; Ho, W.S.; Lee, C.-T.; Hashim, H.; Mansor, N.N.A.; Ho, C.S.; Ramli, A.R.; Takeshi, F. Towards low carbon society in Iskandar Malaysia: Implementation and feasibility of community organic waste composting. *J. Environ. Manag.* **2017**, *203*, 679–687. [CrossRef]
67. Zaman, A.U. A comprehensive study of the environmental and economic benefits of resource recovery from global waste management systems. *J. Clean. Prod.* **2016**, *124*, 41–50. [CrossRef]
68. Pergola, M.; Piccolo, A.; Palese, A.M.; Ingrao, C.; Di Meo, V.; Celano, G. A combined assessment of the energy, economic and environmental issues associated with on-farm manure composting processes: Two case studies in South of Italy. *J. Clean. Prod.* **2018**, *172*, 3969–3981. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.