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. Paolo Fuschi

In copertina
Motta San Giovanni, Azienda Orfei (RC).

Desidero esprimere la mia profonda gratitudine alla Professoressa Adele Muscolo per il suo prezioso supporto e la sua straordinaria competenza, per me Lei è stata un modello di eccellenza da seguire lungo questo percorso. Un sincero ringraziamento va al Professore Giuseppe Celano, che ha creduto in me sin dal primo momento, sostenendo con dedizione le mie ricerche e rappresentando un punto di riferimento fondamentale. Grazie al Coordinatore, Professore Leonardo Schena, e a tutti i docenti dell'Università Mediterranea di Reggio Calabria che hanno contribuito a questo percorso di formazione scientifica ma anche personale. Ringrazio l'azienda agricola Orfei per aver accolto e supportato parte della mia ricerca, e il NIAB (Cambridge, UK) per l'ospitalità e le opportunità di crescita. Grazie ai miei genitori, che con sacrificio e amore hanno piantato in me il seme della conoscenza, nutrendolo con fiducia e cura. Grazie ai miei fratelli, i rami che si sono intrecciati al mio cammino, sostenendomi e crescendo insieme a me. Grazie Tommaso, per essere stato la mia roccia e mai una pietra d'inciampo lungo il mio cammino. A tutti i miei colleghi, con cui ho avuto il privilegio di collaborare: la vostra presenza ha reso questo percorso ancora più significativo!

Note biografiche
Ho conseguito la Laurea Magistrale LM-69 specializzandomi nell'analisi della sostenibilità delle filiere agro-alimentari. La mia ricerca è stata guidata da una profonda sensibilità verso le tematiche ambientali, con un approccio innovativo orientato alla tutela delle risorse naturali. In particolare, ho dedicato il mio impegno scientifico alla salvaguardia del suolo, una risorsa preziosa e fondamentale per l'equilibrio degli ecosistemi e un futuro sostenibile.

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PRODUCTION OF ENVIRONMENTALLY SUSTAINABLE FERTILIZERS FROM AGRO-INDUSTRIAL WASTES FOR THE REHABILITATION OF DEGRADED AREAS AND BIODIVERSITY PROTECTION

Dottorando
Angela Maffia

[redacted]

prof. Adele Maria

[redacted]

prof. Giuseppe
Celano

[redacted]

Co
Dottorato prof.
Leonardo Schena

[redacted]

Table of contents

Abstract/Sommario

The global ecological crisis, manifested by the dramatic loss of biodiversity and progressive soil degradation, imposes urgent and global challenges, with a particularly relevant impact in Calabria, where agriculture is a fundamental pillar of the economy. In this scenario, the management of agro-industrial waste, such as citrus pulp and olive pomace, together with industrial waste, including Sulphur from oil gas desulphurization, contributes to amplifying environmental pressure, further complicating the sustainability of the local production cycle.
This study proposes an innovative approach that applies the principles of the circular economy, in line with Directive 2008/98/EC and EEC Regulation 2092/91, by transforming this waste into environmentally sustainable fertilizers, aligning with the European Green Deal and Farm to Fork strategy objectives to reduce the use of chemical fertilizers and promote recycled resources. The eco-sustainable fertilizers developed were tested and compared with synthetic (NPK) and commercial organic fertilizers typically used by local producers, on typical soils of the Grecanica area (Calabria) and on horticultural crops, such as *Solanum lycopersicum* L. (tomato), *Allium sativum* L. (garlic), and tree crops such as *Corylus avellana* L. (hazelnut). The results on soil fertility showed that eco-fertilizers were highly effective in counteracting soil degradation, generating a significant improvement in aggregate stability and chemical-biological soil properties. An increase in microbial biomass carbon was observed, along with an enhancement of key enzymes for organic matter decomposition, nutrient cycling and overall soil health. These effects far exceeded the performance of commercial synthetic and organic fertilizers. The eco-friendly fertilizers developed also stimulated an increase in microbial biodiversity in the rhizosphere, enriching the soil with beneficial fungi and bacteria essential for nutrient cycling, organic matter decomposition and natural plant defense. Regarding crop quality, eco-sustainable fertilizers have shown superior performance compared to commercial synthetic and organic fertilizers, not only in improving yield, but also in enhancing the nutritional and organoleptic quality of products. Indeed, there was a significant increase in the levels of antioxidants, bioactive compounds and vitamins, crucial factors in improving competitiveness and ensuring the sustainability of local production. In terms of environmental sustainability, assessed according to the life cycle approach (LCA) in accordance with ISO 14044 and ISO 14046, these innovative fertilizers showed a lower carbon and water footprint than conventional synthetic and organic fertilizers, both at the production stage and during application to the soil, with reductions in direct and indirect climate-altering gas emissions. In conclusion, the valorization of agro-industrial wastes as environmentally sustainable fertilizers represents a replicable, low-impact strategy to combat soil degradation and biodiversity loss. This approach is in line with European sustainability regulations and favors the transition to more efficient and environmentally friendly agricultural practices.
Keywords: Organic wastes; Industrial wastes; Soil Fertility; Crop Quality; Circular Economy; Sustainability.

La crisi ecologica globale, manifestata dalla drammatica perdita di biodiversità e dal progressivo degrado del suolo, impone sfide urgenti e globali, con un impatto particolarmente rilevante in Calabria, dove l'agricoltura rappresenta un pilastro fondamentale dell'economia. In questo scenario, la gestione degli scarti agro-industriali, come il pastazzo di agrumi e la sansa di olive, insieme ai rifiuti industriali, tra cui lo zolfo proveniente dalla desolforazione dei gas del petrolio, contribuisce ad amplificare la pressione ambientale, complicando ulteriormente la sostenibilità del ciclo produttivo locale. Questo studio propone un approccio innovativo che applica i principi dell'economia circolare, in linea con la Direttiva 2008/98/CE e il Regolamento CEE 2092/91, trasformando tali rifiuti in fertilizzanti ecosostenibili, allineandosi agli obiettivi europei del Green Deal e della strategia Farm to Fork, volti a ridurre l'uso di fertilizzanti chimici e promuovere risorse riciclate. I fertilizzanti ecosostenibili sviluppati sono stati testati e confrontati con fertilizzanti di sintesi (NPK) e organici commerciali tipicamente utilizzati dai produttori locali, su suoli tipici dell'area Grecanica (Calabria) e su colture orticole, come *Solanum lycopersicum* L. (pomodoro), *Allium sativum* L. (aglio), e colture arboree come *Corylus avellana* L. (nocciolo). I risultati sulla fertilità del suolo hanno evidenziato che i fertilizzanti ecosostenibili sono stati altamente efficaci nel contrastare il degrado del suolo, generando un significativo miglioramento nella stabilità degli aggregati e nelle proprietà chimico-biologiche del suolo. È stato osservato un aumento del carbonio nella biomassa microbica, insieme a un potenziamento degli enzimi chiave per la decomposizione della materia organica, la ciclicità dei nutrienti e la salute complessiva del suolo. Questi effetti hanno superato di gran lunga le performance dei fertilizzanti sintetici e organici commerciali. I fertilizzanti ecosostenibili sviluppati hanno inoltre stimolato un aumento della biodiversità microbica nella rizosfera, arricchendo il suolo di funghi e batteri benefici essenziali per il ciclo dei nutrienti, la decomposizione della materia organica e la difesa naturale delle piante. Per quanto riguarda la qualità delle colture, i fertilizzanti ecosostenibili hanno mostrato una performance superiore rispetto ai fertilizzanti sintetici e organici commerciali, non solo nel migliorare la resa, ma anche nel potenziare la qualità nutrizionale e organolettica dei prodotti. Si è infatti registrato un incremento significativo dei livelli di antiossidanti, composti bioattivi e vitamine, fattori cruciali per migliorare la competitività e garantire la sostenibilità delle produzioni locali. In termini di sostenibilità ambientale, valutata secondo l'approccio del ciclo di vita (LCA) in conformità con le norme ISO 14044 e ISO 14046, questi innovativi fertilizzanti hanno evidenziato un'impronta di carbonio e idrica inferiore rispetto ai fertilizzanti sintetici e organici convenzionali, sia nella fase di produzione che durante l'applicazione al suolo, con riduzioni delle emissioni dirette e indirette di gas clima-alteranti. In conclusione, la valorizzazione dei rifiuti agroindustriali come fertilizzanti ecosostenibili rappresenta una strategia replicabile e a basso impatto per combattere il degrado del suolo e la perdita di biodiversità. Questo approccio è in linea con le normative europee sulla sostenibilità e favorisce la transizione verso pratiche agricole più efficienti e rispettose dell'ambiente.

Parole chiave: Rifiuti organici; Rifiuti industriali; Fertilità del suolo; Qualità delle colture; Economia Circolare; Sostenibilità.

Chapter 1 - Introduction

1.1 Global Challenges for Agriculture

Agriculture is the backbone of food security and the livelihood of billions of people around the world. However, it currently faces several global challenges that threaten not only its productive capacity, but also the health of ecosystems and biodiversity. Intensive agriculture, characterized by high yields, monocultures and extensive use of chemical fertilizers, has shown clear signs of inefficiency, leading to significant losses of microbial biodiversity, biomass and biological activity in agricultural soils, thus compromising soil health (Diacono & Montemurro, 2010; Zhou, 2020).

Soil health, as defined by Doran and Zeiss (2000), refers to '*the ability of soil to function as a viable living system within ecosystem and land-use boundaries*. This concept is fundamental not only for sustaining crop production, but also for maintaining or improving air and water quality. Over the years, the understanding of soil health has evolved significantly. Initially, agricultural research focused mainly on soil quality and crop yields. However, since the 1990s, the emphasis has shifted to soil health as it relates to specific properties and its ability to support long-term ecological functions, thus supporting the development of sustainable agricultural systems. Soil health is thus seen as '*the ability of soil to function effectively by providing ecosystem services'* (Van Es & Karlen, 2019), or its ability to support crop growth without degradation or environmental damage. (Acton & Gregorich, 1995).

The terms 'soil health' and 'soil quality' are often used interchangeably, although they place different emphases on agricultural production and environmental sustainability (Doran, 1996). Farmers prefer the term '*soil health'*, considering soil as a living dynamic entity operating as an integrated system. Scientists, on the contrary, often use '*soil quality*', focusing on the physical, chemical and biological characteristics of the soil. The subjective assessment of soil health poses challenges, as it is difficult to quantify.

In recent years, various factors, including erosion, loss of organic matter, contamination and overuse of chemicals, have undermined soil health. This has reduced the ability of soils to support sustainable agricultural production and provide essential ecosystem services. Soils have become increasingly degraded and less resilient to environmental stresses. According to FAO, about one third of global soils are currently degraded, with negative consequences for both agricultural productivity and environmental health (FAO, 2015). In Europe, particularly in southern Europe, about 25 per cent of the land area, is at high or very high risk of desertification, with an increase of 14 per cent in the last few years (Pravalie, 2017). The main threats to soil in the European Union include erosion, organic matter decline, biodiversity loss, contamination, soil sealing, compaction and salinization (EC, 2006).

The Mediterranean region is highly susceptible to soil degradation (Lahmar & Ruellan, 2007). This area has the highest erosion rates in the EU (Panagos, 2020) and severe salinization problems (Stolte, 2016).

Like reported by Aguilera et al (2013), Mediterranean region has low soil organic matter levels, often below 40 t C ha^{-1} and like reported by Stolte et al (2016), it's because soil organic matter has a negative correlation with temperature and positive correlation with precipitation. In other words, higher temperatures tend to reduce soil organic matter levels, while increased rainfall helps to boost its accumulation. These climatic factors contribute significantly to the relatively low organic matter content observed in the Mediterranean region's soils

In addition, recent studies show that the Mediterranean region faces high surface soil erosion, significant human pressures (Guittonny-Philippe, 2014) and significant vulnerability to climate change (Bourlion & Ferrer, 2018; IPCC, 2019). Italy, with its highly human-modified landscapes and complex morphology, is not immune to these degradation processes. This is particularly evident in the southern regions and islands (Basilicata, Puglia, Calabria, Sardinia and Sicily), where climatic and anthropogenic stresses have caused a sharp decline in agricultural and biological productivity, along with a progressive loss of biodiversity in natural ecosystems (Zambon, 2018). According to Costantini (2013), the annual direct costs of the main soil degradation processes are estimated at more than EUR 38 billion in Europe, while in Italy the costs related to landslides, floods and soil erosion alone amount to EUR 900 million per year. The loss of food production capacity due to soil degradation is particularly alarming in Italy, where food self-sufficiency has recently dropped below 80 per cent (Costantini, 2013). Intensive agriculture and dependence on synthetic fertilizers with urbanization and climatic changes have accelerated soil degradation, with soil quality and biodiversity loss.

Maintaining soil fertility is crucial for sustaining agricultural productivity, and fertilization plays a key role in this process. However, the growing reliance on synthetic fertilizers has raised significant environmental concerns. While fertilizers are essential for providing plants with necessary nutrients, their overuse can lead to the accumulation of pollutants that negatively affect environmental sustainability. As a result, there has been a growing focus on exploring alternative fertilizers that offer minimal environmental impact (Bhardwaj, 2014).

1.2 A Regulatory Framework for sustainable agricultural practices

In 2015, the EU introduced a new economic model under the initiative 'Closing the Loop - An Action Plan for the Circular Economy' (The European Commission, 2015). The goal was to valorize waste by converting it into valuable substrates and raw materials, which could be processed to create innovative new products. This initiative aimed to promote a circular economy, where components with potential value are recovered from agricultural wastes, co-products, and by-products (AWCB), thereby contributing to closing the material loop (Diacono, 2019).

Indeed, in the last decade, interest in renewable biomass feedstocks as a source of agricultural fertilizers was growing (Chew, 2019) and new regulations have also been disseminated for more virtuous agriculture, such as organic farming Regulation (EU) 2018/848 (European Parliament and Council, 2018) on organic production and labeling of organic products. This progress is becoming evident: between 1990 and 2019, Europe saw a 28% reduction in emissions, with declines across all sectors except for transport and air conditioning. The most significant decrease occurred in manufacturing industries. In the agricultural sector, emissions fell by 20.5%, primarily due to a 28% reduction in the cattle population and decreases in the use of synthetic (25%) and organic (13%) fertilizers, as highlighted by the European Environment Agency (European Environment Agency, 2021). The specific sub-sectors of enteric fermentation and direct emissions from agricultural soils contributed to a 4.3% reduction in total emissions between 1990 and 2019 and are the sectors that mostly have reduced their emissions, comparable to the combined reductions achieved by the cement, aluminum, and fluorochemical industries.

Despite this, enteric fermentation still contributes over 48% of all current agricultural emissions, while the remaining emissions in the agricultural sector are mainly related to soil (fertilizer), crop and residue management (Boix-Fayos, 2023) (Figure 1)

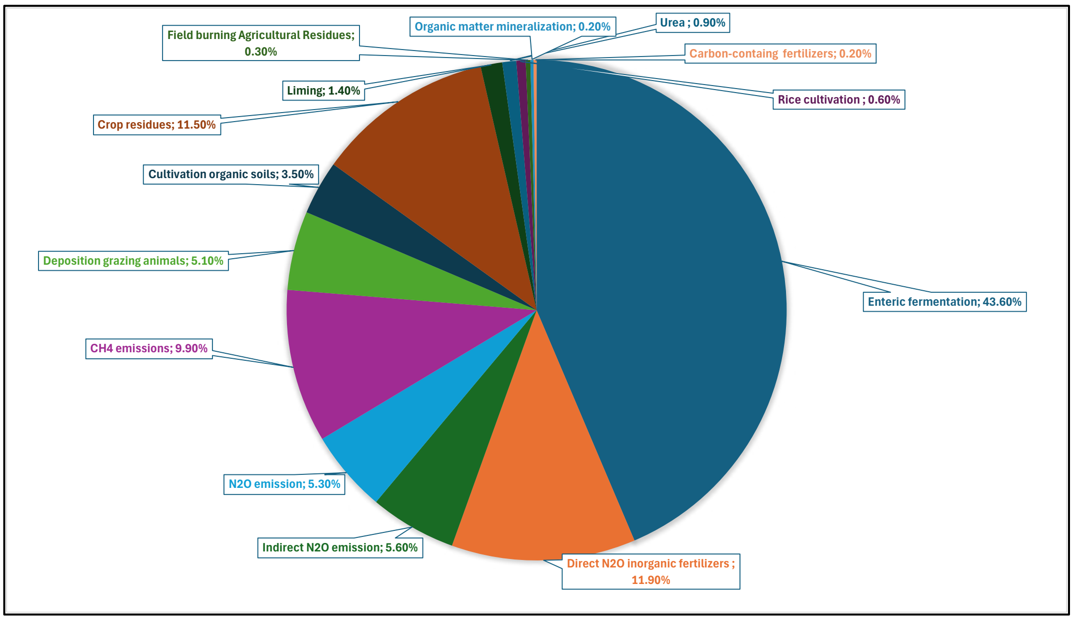

Figure 1. Emissions in the agricultural sector (in % of kt CO_2 eq year^{-1}) in the EU averaged from 2000 to 2018.

(Own elaboration; Data source: European Environmental Agency, 2021).

On 11 December in 2019, the European Green Deal (EGD) initiative is officially presented for the first time, comprising a series of initiatives, strategies and legislative acts that together aim to enable an equitable, sustainable and inclusive transformation of European society and economy. The EDG is the EU's new growth strategy, which "*aims to transform the EU into a fair and prosperous society with competitive economy*" (European Commission, 2020a).

The European Green Deal (EGD) is not a standalone law but rather a comprehensive policy strategy outlining ambitions and objectives across various environmental policy sectors. To implement it, existing regulations and standards will be revised over the coming years, and new laws and directives will be developed and introduced. The Green Deal's framework consists of eight key areas that set a course toward a balanced and sustainable ecological transition depicted in figure 2. These eight objectives are part of a vision for sustainable growth that aims not only to protect the environment but also to create new economic opportunities and improve the quality of life for European citizens.

Figure 2. The European Green Deal and its key areas (Source: European Commission 2020a)

The Farm to Fork (F2F) strategy (European Commission, 2020b, European Commission, 2020c) introduced in 2020, complements the Green Deal by committing to creating fairer and more sustainable food systems across Europe. The strategy sets ambitious goals and aims to promote a transition to greener agriculture, assuming the need to reduce dependence on pesticides, antimicrobials and fertilizers, increase organic farming, improve animal welfare and reverse the loss of biodiversity. The targets, to be achieved by 2030, include:

- the increase of the organic agricultural area in Europe to 25 per cent
- 50% reduction in the use of pesticides
- reduction of fertilizers use by at least 20%.

This strategy aims at a more sustainable food system, although the concept of 'sustainability' remains rather broad and lacks defined boundaries; indeed, some authors point to a lack of clear and specific interpretation, with many objectives not translated into precise actions (Schebesta and Candel, 2020).

The F2F strategy recognizes that the transition will not be possible without a change in eating habits. Therefore, F2F involves the entire value chain, including society and consumption choices, and the role of citizens is crucial in stimulating and supporting this change. Since market demand affects what and how it is produced, extra-farm measures such as responsible consumption and education are also essential to promote sustainable food systems (Morais, 2021).

The F2F identifies four main transitional instruments to achieve its objectives:

1. adaptation of directives (e.g. sustainable use of pesticides);
2. enforcement of environmental and climate regulations (e.g. fertilizer reduction control);
3. development of specific action plans (e.g. action plan for integrated nutrient management and organic farming);
4. adaptation of the new CAP to the objectives of the Green Deal (e.g. inclusion of targeted measures in the CAP national strategic plans.

The Common Agricultural Policy (CAP) is another crucial tool for promoting sustainability in the European agricultural sector. It provides financial incentives and development programmers to encourage farmers to adopt practices that protect soil health and enhance biodiversity. The CAP integrates economic and environmental objectives, focusing on the long-term sustainability of agricultural resources. Complementary to these strategies are concepts such as zero waste and circular economy, both of which are essential to reduce waste and optimize the use of resources (Common Agricultural Policy 2023-2027).

The circular economy can help to restore degraded areas and promote sustainable agricultural practices. The new CAP will be implemented at the national level with national strategic plans (Nadeu, 2023) and instruments focused on achieving the same environmental and climate objectives that contribute to the European Green Deal. In total, 40% of the CAP budget will be climate-relevant (European Court of Auditors, 2021)

Regulation (EU) 2019/1009, known as the Fertilizer Products Regulation (FPR), which came into force in July 2022, is a crucial part of the European Green Deal initiatives aimed at promoting sustainable agriculture and the environmentally friendly management of agricultural waste. This regulation has established uniform standards for the marketing of fertilizer products in the EU, as one of the main objectives of the FPR is to harmonize standards for fertilizer products across the EU. This harmonization facilitates the free movement of CE-labeled fertilizer products within the internal market, allowing them to be marketed in all Member States, provided they meet defined standards of safety, quality and efficacy. This regulation has expanded the scope of permitted products to include those derived from organic waste, recognizing the role of recycled materials in supporting sustainable agriculture.

The key components of Regulation (EU) 2019/1009, depicted in Figure 2, are:

- Product Function Categories (PFCs): The Regulation introduces different PFCs under which different types of fertilizer products can be classified, including:

1. Fertilizers, divided into organic, organic-mineral and inorganic
2. Calcination materials

3. Soil conditioners
4. Culture substrates
5. Inhibitors
6. Plant bio stimulants divided into microbial and non-microbial
7. Fertilizer mixtures

- Categories of component materials (CMCs) define the types of raw materials allowed for the manufacture of fertilizer products. The eleven categories specify the materials acceptable for the manufacture of fertilizers and include:

1) Substances and mixtures of virgin materials: This category includes new materials, not derived from waste or recycling processes. "Virgin" materials include natural minerals, chemical compounds, or purified elements that are directly extracted and processed.
2) Plants, plant parts, or plant extracts: This includes materials derived from plants or specific plant parts, such as leaves or roots, and their extracts, which can contribute beneficial nutrients to the soil.
3) Compost: Materials derived from composting are included but must meet strict standards, as they may contain heavy metals or pathogens that could be harmful if not properly treated.
4) Digestate of fresh crops: This includes digestate obtained through anaerobic digestion of fresh crops, where plant-based organic matter undergoes controlled fermentation.
5) Digestate other than fresh crop digestate: This category covers digestates derived from other organic materials, such as agricultural residues or animal by-products, which must meet specific standards to ensure safety.
6) By-products of the food industry: This includes waste materials derived from food processing, that is, secondary materials from a production process that do not represent a primary product but can offer added value.
7) Microorganisms: This includes beneficial microorganisms, such as nitrogen-fixing bacteria, which help improve nutrient efficiency.
8) Nutritive polymers: These include natural or synthetic polymer compounds that contain or retain nutrients and release them gradually to plants. These polymers must be biodegradable or non-toxic to prevent the accumulation of non-degradable materials in the environment, and nutrient release is regulated to ensure sustainable efficacy.
9) Polymers other than nutrient polymers: This category includes polymers used as binders, coatings, or structural elements in fertilizers without containing nutrients

themselves. For example, biodegradable polymers serve as carriers for nutrients or soil enhancers, promoting controlled release of nutrient substances.

10) Derived products within the meaning of Regulation (EC) No 1069/2009: This category refers to materials derived from animal by-products not intended for human consumption, as outlined in Regulation (EC) No 1069/2009.

11) By-products within the meaning of Directive 2008/98/EC: Defined in the Waste Framework Directive (2008/98/EC), this category includes by-products from industrial processes that are not considered waste but can be used in fertilizers. Examples include materials like ash from biomass combustion or gypsum from industrial processes, which can benefit the soil and must meet quality and safety standards.

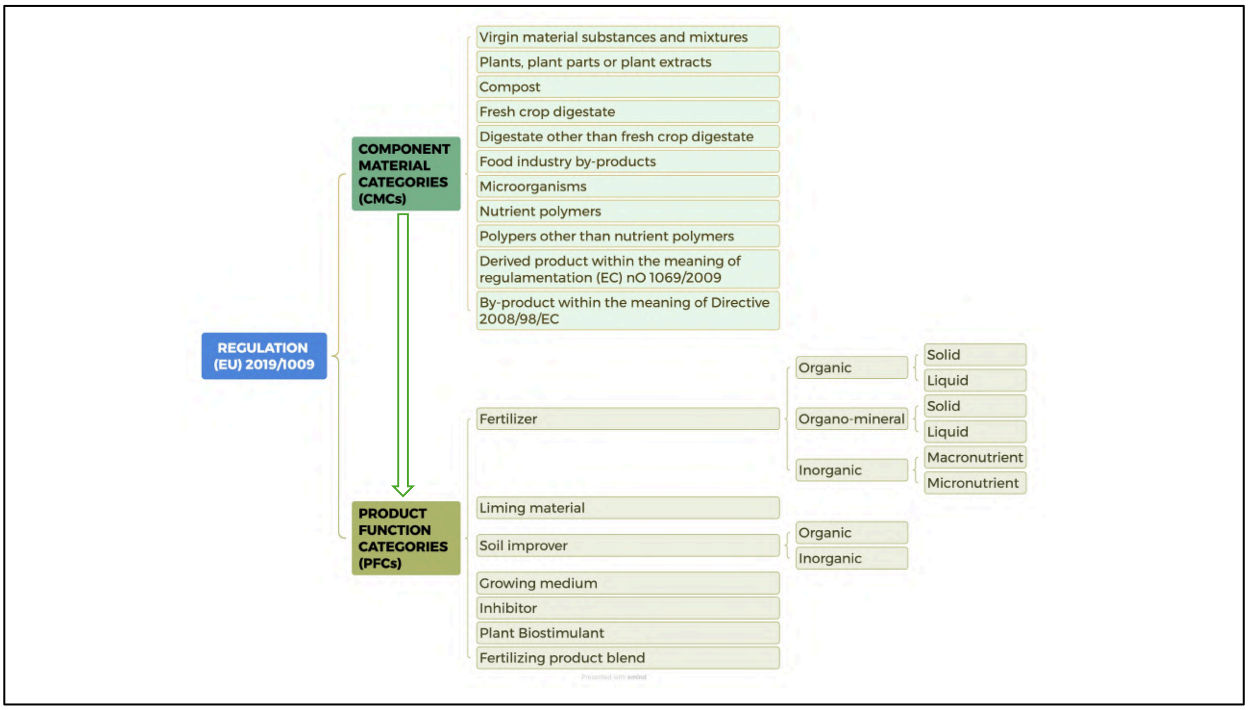

Figure 3. Schematization of the Regulation (EU) 2019/1009 with Product Function Categories (PFCs) derived from Component Material Categories (CMSs). Source: own elaboration.

The decline in soil biodiversity has been identified as one of the major threats and issues to deal with in the coming years (McBratney, 2014). Most European countries have 40 per cent of their soils with a moderately high risk of biodiversity loss, both in terms of microorganisms and the biological functions performed by fauna (Orgiazzi, 2015). (Figure 5). Agricultural soils are particularly threatened, showing the highest percentages in all soil biodiversity categories. (Orgiazzi, 2015)

This situation is worrying because soil biodiversity is essential for numerous ecological processes, such as decomposition of organic matter, regulation of nutrient cycles and support for plant growth (Omer, 2024). Changes in agricultural practices, the intensive use of pesticides and chemical fertilizers, as well as the loss of natural habitats, all contribute to this threat. Consequently, sustainable soil management and the adoption of agricultural practices that protect biodiversity are urgent to ensure the health of agricultural ecosystems and long-term productivity (De Corato, 2023).

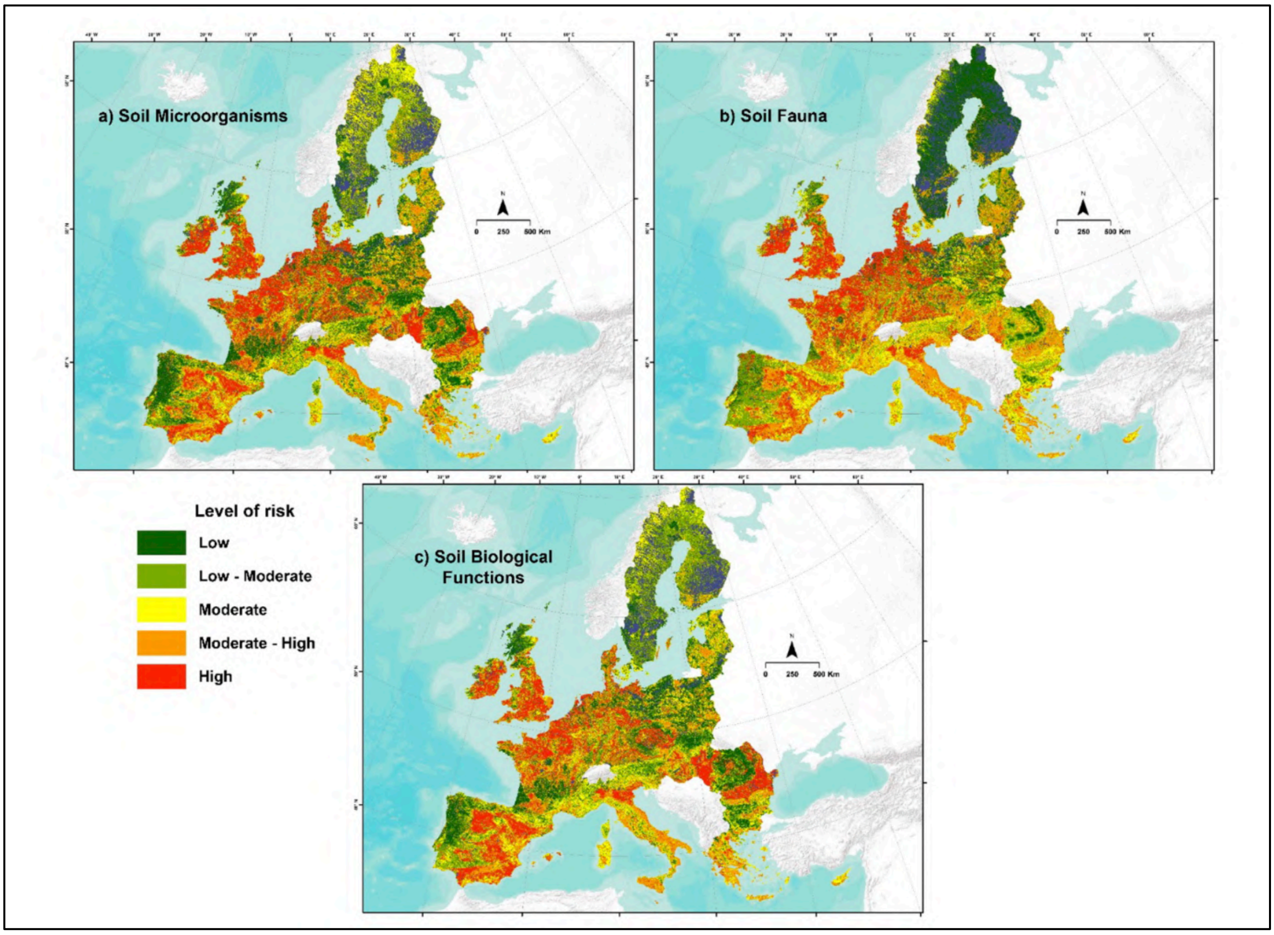

Figure 4. Maps of potential risk to soil biodiversity in Europe. Distribution of the potential threats to (a) soil microorganisms, (b) soil fauna and (c) soil biological functions predicted for 27 European countries (spatial resolution 500 m). (Source: Orgiazzi, 2015)

For this biodiversity is a core pillar of the EGD, recognizing that the health of ecosystems is directly linked to the well-being of people, climate resilience, and economic sustainability. The goal is to halt and reverse biodiversity loss across the continent, as the degradation of natural habitats, the overexploitation of species, pollution, climate change, and the spread of invasive species are putting Europe's ecosystems under unprecedented pressure. Therefore, it will be essential to significantly reduce the use of chemical fertilizers to restore soil fertility and support biodiversity.

1.3 The Impact of Agro-Industrial Waste and Synthetic Fertilizers on Soil and Climate

The growing volume of organic waste, primarily from agro-industrial sources, highlights a significant environmental challenge but also an opportunity for agricultural innovation. With over 1.3 billion tons of organic waste generated globally each year, a figure that could rise to 2.2 billion tons by 2025 (Mehariya, 2018), current waste management practices are struggling to keep pace, leading to adverse effects on soil health, ecosystems, and public health (Sadh, 2018). An innovative approach to managing this waste involves converting it into organic fertilizers. This strategy not only reduces the sheer volume of waste but can also enhance soil fertility, yielding benefits for agricultural productivity. Research suggests that improving organic waste management could potentially reduce agro-industrial waste by 30-40%, potentially increasing global food availability by up to 15% (Wanga, 2016).

However, the environmental impact of agriculture extends beyond waste management. Agriculture is a major contributor to global greenhouse gas emissions, responsible for nearly one-third of total anthropogenic emissions (Crippa, 2021). Transitioning towards a circular approach, where waste products are recycled into valuable agricultural inputs, could play a vital role in reducing both waste and emissions. This holistic approach promises environmental benefits while also addressing food security by sustaining soil health and productivity through waste-based fertilizers.

The rising pressure to ensure food security for a growing global population has driven massive reliance on synthetic fertilizers, valued for their ability to rapidly release nutrients and promote accelerated plant growth. While initially effective in meeting the nutrient demands of soil and crops, their excessive use has revealed significant adverse impacts on the environmental and long-term soil (Sneha, 2018).

In the current climate emergency, the production and application of synthetic fertilizers release CO_2, N_2O, and CH_4, potent greenhouse gasses that significantly contribute to climate change. In the European Union, fertilizer production accounted for approximately 2.5% of global GHG emissions in 2020, and this sector, due to its energy-intensive processes, contributes to 33% of global reactive nitrogen generation (Chehade, 2021). Notably, the synthesis of ammonia, a key component of most synthetic fertilizers, alone consumes 2% of global energy and accounts for 0.8% of global GHG emissions (International Energy Agency, 2021).

Agricultural intensification and widespread overuse of synthetic fertilizers have also led to increased contamination of aquatic and terrestrial ecosystems due to nutrient runoff (Xu, 2022), triggering eutrophication and biodiversity loss (Wang, 2022). At the same time, the recent spike in fertilizer prices, driven in part by geopolitical factors like the war in Ukraine, has intensified challenges for farmers and raised serious concerns about global food security (Arndt, 2024).

In response to these interconnected challenges, the European Union has initiated a plan to promote circular economy principles in agriculture, encouraging the use of residual biomass as a raw material for fertilizer production. This approach aims to close nutrient cycles, reduce pollutant dispersion, and enhance agricultural sustainability while decreasing reliance on synthetic fertilizers (Scholtz, 2017).

Awareness is growing around the need for organic fertilizers and more sustainable agricultural practices to mitigate environmental and climate impacts. Organic fertilizers derived from agro-industrial waste can contribute to reducing GHG emissions, improve nitrogen use efficiency (NUE), and lessen dependence on synthetic inputs (Linquist, 2012; Chivenge, 2011). These organic fertilizers not only alleviate environmental impacts but are also pivotal in the transition to regenerative agriculture that prioritizes soil health and lessens dependence on fossil resources (Christel, 2014).

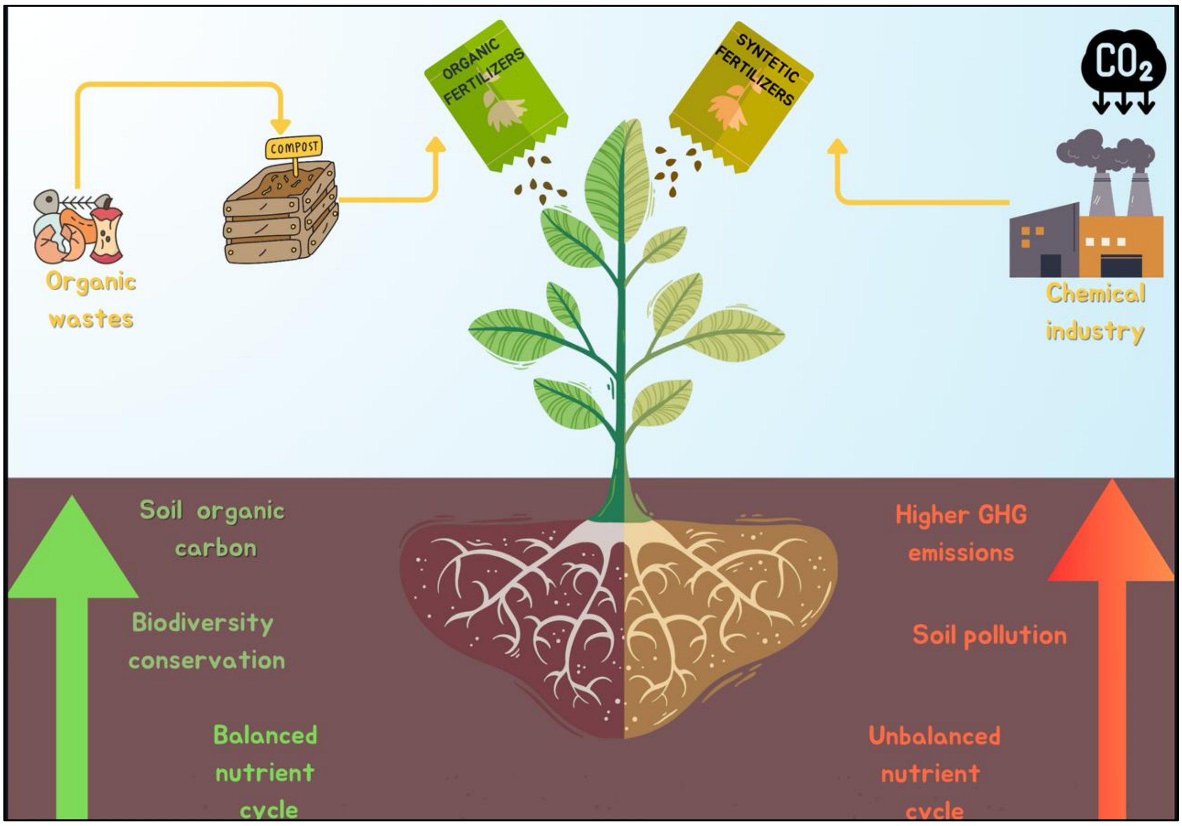

Figure 5. Effect of organic and synthetic fertilizers on soil and climate. Source: own elaboration.

1.4 Waste Valorization Techniques for agricultural proposes

There are several techniques to transform organic waste into a resource, among them composting, vermicomposting and anaerobic digestion are the most used processes (Lohri, 2017) In composting, organic materials of plant and animal origin undergo degradation through a controlled process driven by a succession of microbial communities under aerobic conditions. This process involves an increase in temperature, promoting the transformation of organic matter into a stabilized and humified product known as compost (Pergola 2018; Sharma, 2020), in a reasonably short time (a few weeks) (Vallini, 1995).

Different methods are used to perform composting procedures. For this reason, there are different types of composting systems such as conventional windrow, aerated static pile, in-vessel and the newly developed two-stage composting system (Bernal, 2009, Lim Y.L. 2017). It is important to emphasize that many parameters such as temperature, pH, oxygen, porosity, moisture content, etc., must be checked throughout the process and constantly optimized to achieve a good composting result (Bian, 2019; Sayara, 2020; Palese, 2020).

As reported by several authors, the process is divided into an initial period of intense decay (mesophilic phase) carried out by the presence of mesophilic microbial preconceptions that utilize the bioavailable compounds of the starting matrices. This phase produces a lot of heat as the activity of the micro-organisms heats up the matrix, whereby the 45 °C temperature is exceeded, and the mesophilic micro-organisms are replaced by thermophilic ones that can also work at temperatures of more than 70°C. This thermophilic phase is followed by a maturation period, corresponding to the mesophilic maturation phase (Makan, 2013; Mustin, 1987, Fourti, 2013; Oviedo-Ocaña, 2015). Compost, like most recycled biomass and organic matter, is characterized by a complex composition and an extensive diversity of microorganisms. This complexity and variability make it impossible to establish universal quality standards for the various types of compost. Current regulations (Legislative Decree 75/2010) set maximum allowable levels for potentially toxic substances and define optimal ranges for key components, such as the C/N ratio, pH, moisture content, and the presence of essential microorganisms. For this reason, the scientific community has been working for years to determine additional quality parameters that ensure high-quality compost with beneficial effects on soil and plants. For example, a study of Bernal et al (2017) showed that the cation exchange capacity and cation exchange capacity/Organic carbon can also be used as an indicator for the maturity of compost. A different study showed that respiration indices are very useful for evaluating the stability of final products such as stabilized material for landfill or compost (Adani, 2006; Berrena, 2014). A study of Ameen et al (2016) showed that pH level of composting materials impacts the rate of the process. An alkaline pH is generally preferred for efficient composting, whereas an acidic pH slows down the process by damaging the microorganisms responsible for decomposition. If all the parameters that enable the identification of quality compost are correctly monitored, quality compost can eventually be obtained that, thanks to the ample nutrients found in composted materials and the presence of beneficial microorganisms, promote plant growth, agricultural productivity and organic matter content of the soils (Luo, 2017; Pane, 2014).

Vermicomposting, like traditional composting, is a process of controlled decomposition of organic matter; however, it is distinguished by the fact that earthworms play a central role by actively participating in the early stages of decomposition, significantly accelerating waste stabilization (Alshehrei and Ameen, 2021). Epigeous earthworms, which naturally inhabit the surface layers of litter, are typically selected for vermicomposting because of their rapid reproduction rates and high consumption of organic matter (Blouin, 2013).

Earthworms break down organic matter and change its physical, chemical and biological state and gradually reduce by reducing the C:N ratio ratio (Domínguez, 2017).

In addition, earthworms greatly improve soil fertility, and the result is a large amount of mineralized N that is more available to plants. After earthworm rearing, there is an increase in nitrogen in the soil (Edwards, 1976; Ruz-Jarez 1992; Ozawa 2005), as the earthworm body comprises 3% ash, 14% fat, 14% carbohydrate and 65% protein (Govindan VS, 1988). When an earthworm dies, about 0.01 g of nitrate comes into the soil and 72% of its dry weight is protein (Ronald, 1977). One study observed that conventional compost is richer in 'ammonium', while vermicompost tends to be richer in 'nitrate', which is the most bioavailable form of nitrogen for plants (Atiyeh, 2020). It has also been claimed that vermicompost has a higher N availability than conventional compost on a weight basis and the supply of several other plant nutrients, such as phosphorus (P), potassium (K) and magnesium (Mg). (Takur, 2021).

Anaerobic digestion (AD) is another biochemical process in which a series of microbial reactions convert solid organic matter into biogas, mainly methane (CH_4) and carbon dioxide (CO_2), along with small amounts of other gasses such as hydrogen (H_2), ammonia (NH_3), hydrogen sulphide (H_2S) and nitrogen (N_2) (Chatterjee and Mazumder, 2016). The process also produces a by-product known as digestate, a nutrient-rich slurry with a solid content of generally less than 6 per cent. Once separated into solid and liquid phases using a decanter or separator, both phases of digestate can be used for agricultural purposes, producing anaerobic digestate (Bauer, 2009; Nkoa, 2013) or as a renewable energy source (biogas).

As investigated by several studies (Provenzano, 2011; Teglia, 2011; Furukawa and Hasegawa, 2006; Voća, 2005; Möller, 2008), anaerobic digestates inherit the chemical attributes of the organic matrix from which they were produced. Thus, several cited studies show ranges of variation in organic matter content of 38-75%, cellulose/lignin ratio (0.22 -1.75), oxygen uptake rate (1.129 - 3.774) and C/N ratio (6.2 - 24.8).

To date, composting technologies and the vermicomposting and aerobic digestion of organic waste are widely studied and are becoming common practices for the proper management of agricultural waste, also due to the low environmental impacts associated with their production when compared to the impacts of traditional fertilizers.

Traditional synthetic fertilizers have a carbon footprint ranging from a minimum of 1816 kg CO_2 eq per ton of fertilizer to a maximum of 2107.99 kg per ton, as demonstrated by a recent study by El Chami et al., (2023). Studies assessing the environmental impacts of various organic fertilizers produced through composting, vermicomposting, and anaerobic digestion indicate that the global warming impact ranges from 130 to 448 kg CO_2 eq per ton of fertilizer produced (Mu, 2017; Martinez-Blanco, 2020; Blenghini, 2008). Furthermore, several studies have shown the benefits of fertilizers derived from composting, vermicomposting, and anaerobic digestion in improving key soil properties, such as structure, cation exchange capacity, microbial activity (Erana, 2019), nutrient availability, and increased microbial biodiversity, which is essential for soil health

(Dotaniya, 2016; Sharma & Garg, 2019; Zhen, 2014) and agricultural productivity.

1.5 From agro-industrial wastes to resources

Italy is one of the world's largest olive producers, with an annual production of about 3.5 million tons, resulting in over 2,000 tons of olive oil residues (Prosodol, 2012).

Among these by-products, olive pomace (OP) stands out as a major organic waste generated during the oil extraction process, especially in the two-phase centrifugation systems widely used in Mediterranean countries, which account for over 97% of global olive production (FAOSTAT, 2024). Produced in large quantities during the short harvest season, generally from October to November, olive pomace is a semi-solid material rich in organic and inorganic compounds, with an organic matter content of around 90%. Managing it poses an environmental challenge due to its phytotoxic and antimicrobial compounds, such as polyphenols and lipids, which could harm the environment if not properly treated (Doula, 2012; Ashraf, 2014).

A sustainable solution is the composting of olive pomace, which allows for the management of large volumes of waste by transforming them into valuable biofertilizers for soil health. This process, which combines pre-treated pomace with other plant residues on-site, produces a stable and mature compost ideal for agricultural use. Composting reduces the carbon-to-nitrogen (C/N) ratio of the pomace, improving microbial activity and accelerating decomposition (Mandal, 2016; Zoghlami, 2016). The use of composted olive pomace offers significant benefits, including improved soil structure, increased cation exchange capacity, and enhanced microbial activity, all of which are crucial for maintaining soil fertility and productivity (Erana, 2019; Das & Dkhar, 2012). Additionally, olive pomace compost facilitates nutrient availability, improves soil aeration, and helps balance pH, promoting crop growth and greater resilience (Raju, 2016; Rekha, 2018). Biologically, the application of olive pomace compost enhances microbial diversity and biomass, particularly in calcareous and alkaline soils, improving nutrient cycling and organic matter mineralization, making essential elements such as carbon and phosphorus available to plants (Sharma & Garg, 2019; Dotaniya, 2016). These improvements contribute to the long-term sustainability of agricultural systems, promoting soil health and greater resilience to environmental stresses (Zhen, 2014; Nayak, 2007).

In addition to being a major olive producer, Italy processes approximately 800,000 tons of oranges annually, generating around 500,000 tons of agro-industrial waste from juice and other citrus-based products (Schmid, 2019). This waste consists mainly of peels, seeds, and pulp, representing a potential source of environmental pollution and greenhouse gas emissions if not properly managed. Ineffective degradation of orange residues can lead to soil and groundwater contamination and produce methane during decomposition, a greenhouse gas that contributes to climate change (Sadh, 2018).

To address these risks and harness the agronomic benefits of orange waste, innovative recycling methods have been developed. One of the most effective approaches is converting orange residues into organic fertilizers through composting and bioconversion processes. This method not only reduces waste volume but also enriches the soil with essential nutrients. Orange residues contain high levels of organic matter, nutrients, organic acids, and beneficial compounds that enhance soil fertility and support plant growth (Ashokkumar, 2022; Hossain, 2017). Composting orange waste with other organic materials can improve the overall quality of compost, producing a nutrient-rich amendment that boosts soil health. The organic acids released during decomposition increase phosphorus availability, a crucial element for plant development (Dotaniya, 2016). The application of compost derived from orange waste also enhances soil structure, increases microbial activity, and promotes water retention, leading to higher crop yields and optimized resilience against environmental stresses (Rekha, 2018).

A study of Consoli et al (2022), confirms that the use of citrus waste as an organic fertilizer offers a great opportunity for the sustainable fruit production because citrus wastes improve soil chemical properties by increasing the cation exchange capacity, retaining water, increasing soil aeration and promoting microbial activity.

In addition to organic agricultural waste, the industrial sector generates significant amounts of by-products that can be recycled and repurposed as valuable resources. A notable example is sulfur, a by-product of crude oil refining. If not properly managed, sulfur released during the refining process can have severe environmental impacts, including acid rain, soil, water, and air contamination, as well as climate change, reducing the quality of life and posing health risks (Al-Bidry & Azeez, 2020).

Sulfur has been recognized for its agricultural value for over a century (Bogdanov, 1899; Hart & Peterson, 1911). Historically, most of the sulfur needed by plants came from atmospheric deposition of SO_2, which was absorbed directly by plants or incorporated into the soil's mineral pool. However, the implementation of air quality standards since the 1990s has significantly reduced sulfur emissions from industrial sources, resulting in decreased sulfur availability in soils (Degryse, 2016; Haneklaus, 2008). Consequently, sulfur deficiency has become a widespread problem, particularly in agricultural soils. This deficiency can be addressed by applying sulfur-based fertilizers, such as sulfate, which is easily available to plants, or elemental sulfur, which requires oxidation in the soil to become accessible (Degryse, 2016).

Another important aspect is sulfuric acid production, which uses over 90% of the recovered sulfur. Although sulfuric acid is essential for agricultural purposes, its production process generates harmful emissions, particularly CO_2. A life cycle assessment study showed that producing one ton of sulfuric acid results in 83.26 kg of CO_2 emissions, highlighting the energy-intensive and unsustainable nature of the process (Marwa, 2017). Nonetheless, sulfur continues to play a crucial role in maintaining soil fertility, especially in preventing sulfur deficiency, which is increasingly widespread (Haneklaus, 2007).

From an industrial perspective, removing sulfur during refining is essential to prevent catalyst deactivation and corrosion of refining equipment. Furthermore, sulfur remaining in fuels can lead to the release of toxic gasses such as hydrogen sulfide (H_2S) and sulfur dioxide (SO_2), worsening air pollution and contributing to acid rain (Saleh, 2020). For these reasons, desulfurization is a key operation in oil refineries, driven by stricter environmental regulations and the global push for cleaner fuels (Saleh, 2020).

Globally, over 80% of industrial sulfur comes from oil and natural gas, which typically contain between 1% and 3% sulfur by weight (Speight, 2019). Removing sulfur during refining is necessary to prevent pollution and enable the use of noble metal catalysts in subsequent refining processes. However, as the world moves toward decarbonization, sulfur supplies from fossil fuels are expected to decrease just as demand for this material increases, particularly for sulfuric acid used in green technologies such as battery production and fertilizers. This could drive up sulfur prices, potentially increasing food production costs (Grennfelt, 2020; IPCC, 2018).

In the United States, the production of elemental sulfur from oil refineries and natural gas treatment plants was estimated at 8.2 million tons in 2019, with over 55% of domestic production concentrated in Louisiana and Texas (US Geological Survey, 2020). Additionally, sulfuric acid recovered from oil refining processes significantly contributes to sulfur recycling, with 2.5–5 million tons of spent sulfuric acid recovered annually (US Geological Survey, 2020).

In agriculture, elemental sulfur is insoluble and requires microbial oxidation to sulfate before it can be absorbed by plants. For this reason, it is commonly used in combination with bentonite clay, which constitutes about 10% of the mixture, to form pellets. When the clay absorbs moisture from the soil, it expands and breaks the pellets into smaller fragments, increasing the reactive surface area and facilitating rapid solubilization (Mikkelsen, 2013). Bentonite, a secondary silicate mineral (1:2), is particularly valued for its ability to expand during hydration and dehydration, increasing its volume up to 15 times. This expansion promotes the rapid solubilization of sulfur (Akkuzu, 2016), in addition to improving the cation exchange capacity

(Mumpton, 1999). Due to these characteristics, bentonite has proven particularly useful in agriculture, either as granules or tablets, to optimize nutrient uptake by plants (Hilal, 2013; Messick, 2005).

Recent studies have confirmed the effectiveness of using waste sulfur. A study of Lisowska et al (2022), observed a significant increase in sulfate content in the soil with the application of waste sulfur, though they highlighted the need to consider the potential acidification effect on the soil. Study of Zhu et al (2010) found that sulfur application increased the levels of enzymes involved in the ascorbate-glutathione antioxidant cycle, delaying plant senescence and enhancing antioxidant activity during the later stages of growth. Additionally, Tabak M et al (2022) suggest that using sulfur-rich waste materials, particularly those generated during gas desulfurization, can be used as an alternative to conventional mineral fertilizers.

The use of agro-industrial wastes and industrial by-products as sustainable fertilizers represents a significant opportunity to rethink agricultural management from a circular economy perspective (Muscolo, 2021). This approach not only reduces wastes but also allows the recovery of nutrient-rich materials, such as olive pomace and citrus processing residues, which can enhance soil fertility. Similarly, recycling sulfur and using it in agriculture, in combination with bentonite, offers new solutions for more eco-friendly fertilization, promoting long-term resilience and sustainability of agricultural soils.

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PhD thesis Objectives

The growing demand for sustainable agricultural practices is essential to counter environmental degradation and the decline in soil health, especially in Mediterranean regions where calcareous-alkaline soils restrict the availability of key macro- and micronutrients for plants (Bauer and Velde, 2014). These challenging soil conditions call for innovative soil management strategies, particularly the use of sulfur, which can effectively acidify alkaline soils and improve nutrient accessibility (Besharati, 2017; Bharathi and Poongothai 2008; Cui, 2004; Kayser, 2000; Safaa and Hanan 2013; Sayahi and Souri 2019). The objective of this doctoral research is to develop and evaluate environmentally sustainable fertilizers derived from agro-industrial wastes, with a focus on utilizing sulfur—a byproduct of industrial processes—and organic residues from the orange and olive processing industries. Addressing the specific agricultural challenges of Calabria, where alkaline soils often result in nutrient deficiencies and hinder crop productivity, this research aims to create innovative fertilizers that improve soil health and support sustainable crop growth.

Through a series of field and pot experiments, the study assesses the effectiveness of these newly developed fertilizers across different soils typical of Calabria's Grecanica area and crops, including tomatoes (*Solanum lycopersicum* L.), garlic (*Allium sativum* L.) and hazelnut (*Corylus avellana* L.). By comparing the effects of these sulfur-enhanced organic fertilizers to conventional chemical fertilizers (NPK) and traditional organic amendments like horse manure, the research investigates their potential to enhance nutrient availability, improve soil properties, and promote sustainable agricultural practices.

The specific objectives of this research are:

1. **Sustainable fertilizer production by agro-industrial wastes:** To evaluate the effectiveness of different waste processing methods (aerobic, anaerobic digestion and crude waste pelleting) in converting olive pomace and orange waste into organic fertilizers. The goal is to check if there is a biomass method specificity and to determine which waste processing method produces fertilizers with better chemical and physical characteristics. This investigation is key to understanding how processing methods and the type of organic matrix influence the agronomic value of waste-derived fertilizers. By identifying the most effective processing methods, the study seeks to provide practical

solutions to producers who face difficulties in managing these byproducts, particularly in Calabria, where large quantities of these wastes are generated. This approach not only supports sustainable agricultural practices, but also contributes to the region's economic development by promoting a circular economy.

2. **Soil fertility improvement**: to rehabilitate degraded soils through the implementation of innovative strategies that enhance soil fertility, increase organic matter content, and improve nutrient bioavailability. Degraded soils frequently exhibit diminished structural integrity, reduced microbial diversity, and lower nutrient-holding capacity, which collectively undermine agricultural productivity. This objective specifically targets the challenges posed by alkaline soil conditions prevalent in Calabria, employing sulfur-rich fertilizers derived from agro-industrial wastes to address these issues. The incorporation of sulfur-rich fertilizers is known to lowering the pH which in turn can enhance the solubility of essential nutrients, facilitating their uptake by plants (Sarma, 2024). In addition, the addition of fertilizers rich in organic matter derived from organic waste contributes to the goal of improving soil fertility by enriching it with essential organic matter (Muscolo, 2022).

   Another important aspect is the promotion of biodiversity within the rhizosphere, which is the zone of soil surrounding plant roots where complex interactions occur between roots, soil, and microorganisms. A rich microbial diversity within the rhizosphere enhances soil resilience, allowing ecosystems to better withstand environmental stresses and adapt to changing conditions (Prashar, 2013)

   A key challenge in creating a sustainable soil environment that supports plant health and productivity is fostering a balanced and thriving microbial ecosystem.

3. **Crop quality enhancement**: To evaluate the effects of the newly developed fertilizers on crops to identify changes in key parameters such as antioxidant properties, vitamin content, and overall nutritional value. The enhancement of crop quality is critical not only for consumer satisfaction but also for promoting public health through the consumption of nutrient-dense foods. The focus on antioxidant properties is essential, as these compounds play a pivotal role in protecting human health by neutralizing free radicals and reducing oxidative stress. (Parcheta, 2021). By analyzing the levels of bioactive compounds, such as polyphenols and flavonoids, this research seeks to determine whether the application of sustainable fertilizers can enhance the natural antioxidant capacity of crops, thereby increasing their health benefits for consumers. In addition to antioxidants, this objective will investigate the impact of these fertilizers on the vitamin

content of the crops. Vitamins are vital micronutrients that support various physiological functions in humans (Godswill, 2020). The relationship between soil health, fertilizer application, and vitamin biosynthesis in plants is an important area of exploration, as improved soil conditions may enhance the metabolic pathways involved in vitamin production. This aspect of the research will evaluate whether the use of sulfur-rich fertilizers can lead to increased concentrations of essential vitamins, thereby contributing to improved nutritional profiles of the crops. Furthermore, this research will assess the overall nutritional value of the crops produced, including macro- and micronutrient content. The sustainable fertilizers developed in this study are designed to not only support robust plant growth but also enhance the nutritional density of the harvested produce. This focus on nutritional value aligns with growing consumer preferences for healthy, sustainably produced food options (Van Bussel, 2022) Ultimately, this objective aims to establish a comprehensive understanding of how the application of environmentally sustainable fertilizers can enhance crop quality, thereby making them more appealing to consumers. By demonstrating the benefits of sustainable agriculture practices, this research aspires to promote the adoption of such methods within the agricultural community, thereby supporting both economic viability and public health objectives

4. **Environmental sustainability**: To conduct a comprehensive environmental impact assessment of the proposed fertilizers using Life Cycle Assessment (LCA) methodology (UNI EN ISO 14040; UNI EN ISO 14044). This objective aims to evaluate the environmental footprint of these fertilizers throughout their life cycle, including production, application, and long-term effects on soil health and ecosystem sustainability. This assessment will encompass multiple stages, including the resource inputs required for fertilizer production, the emissions and waste generated during manufacturing, and the potential environmental consequences associated with their application in agricultural systems. By analyzing these phases, this research aims to provide a holistic view of the environmental impacts associated with the use of these fertilizers. By identifying both the potential environmental benefits and different fertilizer obtained from agro-industrial wastes, this research seeks to contribute valuable insights into the sustainable management of agricultural systems in Calabria. The findings will inform stakeholders, including farmers, policymakers, and agricultural practitioners, about the ecological implications of adopting these innovative fertilizers. Ultimately, this objective aspires to support the development of environmentally sound

agricultural practices that align with the goals of sustainability and resource conservation.

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Results organization

This PhD thesis consists of a collection of 6 scientific papers focused on the production and evaluation of sustainable fertilizers derived from agro-industrial waste. The articles are presented in a logical sequence to ensure a coherent and comprehensive exploration of these interconnected topics.

The first article evaluates three waste transformation processes (aerobic digestion, anaerobic digestion, and crude waste pelleting) using olive pomace and orange waste for verify if the three methods were equally and universally applicable to different biomasses and assess if the efficacy/efficiency of the fertilizers produced on soil properties, growth and antioxidant properties of garlic (*allium sativum*) depended on the processes or on the own chemical characteristics.

The second study assesses the effectiveness of a sustainable fertilizer derived from agro-industrial waste, focusing on sulfur and orange residues at different concentration and compares its impact on the chemical and biological properties of two different soils against synthetic and commercial organic fertilizers. The third study carried out during the research period at the Orfei Agricultural Company (Motta San Giovanni, RC), aims to evaluate the effects of agro-industrial waste-based fertilizer (orange waste + sulfur bentonite) on the quality, Carbon Footprint and Water Footprint of two soils used for growing industrial tomatoes (var. *Big Rio F1*), compared to commonly used chemical (NPK) and organic fertilizers (Horse manure).

The fourth study pursues two primary objectives: to assess how the use of sulfur bentonite in conjunction with orange residue as a bio stimulant influences tomato quality and its antioxidant systems and to investigate both the qualitative and quantitative alterations in the volatile compounds responsible for tomato aroma induced by sulfur bentonite, in comparison to horse manure and NPK fertilizer.

The fifth study discusses the potential and challenges associated with the use of waste-based fertilizers, especially olive pomace, considering both the environmental benefits and the practical difficulties related to the variability of raw materials and the complexity of their management. The aims are 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 and to assess the effectiveness of compost as a soil amendment as well as to evaluate the overall impact of

compost on soil and the environment using the life cycle assessment (LCA) methodology.
The sixth study is about research carried on during the fellowship at the NIAB (Cambridge, UK), employs a metagenomic approach to explore the short-term impact of organic fertilizers, composed with or without sulfur on rhizosphere microbiome biodiversity of 2-year-old *Tonda di Giffoni* hazelnut saplings.

Papers details

Below are the details of the papers highlighting each with its status location and its location in terms of bibliometric indexes.

1) Panuccio Maria Rosaria, Marra Federica, **Maffia Angela**, Mallamaci Carmelo and Muscolo Adele, (2022). *Recycling of agricultural (orange and olive) bio-wastes into ecofriendly fertilizers for improving soil and garlic quality.* Resources Conservation & Recycling Advances https://doi.org/10.1016/j.rcradv.2022.200083.

   Journal bibliometric classification of Resources Conservation & Recycling Advances; IF 5.4, category Waste Management and Disposal quartile Q1.

   Angela Maffia role and contribution of this paper: Data curation, writing original draft preparation, Writing review and editing.

2) Marra Federica, **Maffia Angela**, Canino Francesco, Greco Carmelo, Mallamaci Carmelo and Muscolo Adele, (2023). *Effects of fertilizer produced from agro-industrial wastes on the quality of two different soils*. Archives of Agronomy and Soil Science. https://doi.org/10.1080/03650340.2023.2266218.

   Journal bibliometric classification of Archives of Agronomy and Soil IF 2.3, category Agronomy and Crop Science quartile Q1.

   Angela Maffia role and contribution of this paper: worked in the laboratory, carrying on soil chemical analyses.

3) **Maffia Angela,** Marra Federica, Canino Francesco, Oliva Mariateresa, Mallamaci Carmelo, Celano Giuseppe and Muscolo Adele, (2023). *A comparative Study of Fertilizers in Tomato-Grown Soils: Soil Quality, Sustainability and Carbon/Water Footprint. Soil System*. https://doi.org/10.3390/soilsystems7040109

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

   Angela Maffia role and contribution of this paper: Conceptualization, Software, Methodology.

4) Russo Mariateresa, Di Sanzo Rosa, Marra Federica, Carabetta Sonia, **Maffia Angela**, Mallamaci Carmelo and Muscolo Adele, (2023). *Waste-Derived fertilizer acts as biostimulant, boosting tomato quality and aroma.* Agronomy. https://doi.org/10.3390/agronomy13122854

   Journal bibliometric classification of Agronomy IF 3.3, category Agronomy and Crop science quartile Q1.

   Angela Maffia role and contribution of this paper: software and investigation.

5) **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.

   Angela Maffia role and contribution of this paper: Writing-original Draft; Writing -review and editing, software, methodology.

6) **Maffia Angela**, Scotti Riccardo, Wood Thomas, Muscolo Adele, Lepore Alessandra, Acocella Elisabetta, Celano Giuseppe, (2024).

   Transforming agricultural and Sulfur Wastes into Fertilizer: Assessing Short-Term Effects on Microbial Biodiversity via a Metagenomic Approach. Life

   https://doi.org/10.3390/life14121633

   Journal bibliometric classification of LIFE IF 3.2, category Ecology quartile Q2.

   Angela Maffia role and contribution of this paper: Conceptualization, methodology, software, formal analysis, resources, writing—original draft preparation.

Additional papers:

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

   Influence of agro-industrial waste compost 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.

   Angela Maffia role and contribution of this paper: conceptualization, methodology, software, writing-review and editing.

8) **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

   Angela Maffia role and contribution in this paper: writing—original draft preparation, statistical analysis, formal analysis.

Chapter 2 - Recycling of agricultural (orange and olive) bio-wastes into ecofriendly fertilizers for improving soil and garlic quality.

Panuccio Maria Rosaria[1], Marra Federica[1], Maffia Angela[1], Mallamaci Carmelo[1] and Muscolo Adele[1].

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

Correspondence: amuscolo@unirc.it

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Recycling of agricultural (orange and olive) bio-wastes into ecofriendly fertilizers for improving soil and garlic quality

Panuccio MR [a], Marra F [a], Maffia A [a], Mallamaci C [a], Muscolo A [a,*]

[a] *Department of AGRARIA, Mediterranea University, Feo di Vito, 89122 Reggio Calabria, Italy*

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ABSTRACT

Agricultural waste material even if free from toxic compounds or pathogens can cause environmental problems and their unsustainable use can lead to health and environmental risks. Orange and olive food processing wastes are rich in chemical compounds and could offer many opportunities of use, especially for the high level of nutritional components. This paper proposes to validate anaerobic digestion, aerobic digestion, and raw agricultural waste management as possible ecofriendly methods to turn these wastes into fertilizers. The byproducts obtained by these three different processes, have been chemically analyzed and assessed on soil, growth and antioxidant properties of garlic (*Allium sativum*). Results evidenced that the chemical properties of the soil treated with all the byproducts were positively influenced, even if the effects were different and depended on the type of the byproduct used and on the organic wastes from which the byproducts came from. The byproducts coming from orange wastes (pastazzo) were a bit more effective than those coming from olive pomace and among the byproducts the compost was the best one. Results evidenced that garlic increased its growth and antioxidant capacity when cultivated with all byproducts. The results of this study evidenced that all the byproducts obtained can be used in agriculture with success and the transformation methods used even if differently, are environmentally, economically and/or agriculturally valid.

1. Introduction

Agricultural production is considerably increased over the last 50 years due to the extension of cultivated lands, the increase in technology to enhance the productiveness, and the rise of world population (FAO, 2017; OECD/FAO, 2019). Agriculture is a sector that produces about 23.7 million food tons per day over the world contributing for more than 21% to greenhouse gases emissions (Gerber et al., 2013). The increase in agricultural production is influencing the environment, affecting negatively soil, air and water resources. Nowadays, the new global challenge is to reduce the environmental degradation adopting more ambitious and rapid measures to achieve, in the next 10 years, the goals established by the United Nations 2019 (Global Sustainable Development Report, 2019). EU Green Deal policy set out the trajectory to be climate neutral by 2050. As a milestone towards this target, the EU Commission proposed a 2030 target to reduce greenhouse gas emissions by 55 per cent compared to 1990. The European Green Deal aims to boost the efficient use of resources by moving to a clean, circular economy and stop climate change, revert biodiversity loss and cut pollution, by reducing, reusing and recycling (EU, 2020). The required measures have to consider the criteria of sustainability, focusing mainly on: an efficient reuse of wastes, a lower emission of polluting gases and a reuse of solid wastes (EC, 2019; Ferronato and Torretta, 2019; O'Connor, 2021).

Italy is the second largest European orange and olive producer after Spain, and processes approximately 800 000 tons per year (tpy) of orange, with a waste production of approximately 500 000 tpy, and 3.500.000 tpy of olives with a production of more than 2000 tpy of olive oil wastes (Prosodol, 2012). Orange and olive food processing wastes even if free from toxic elements or pathogens can negatively affect the environment for their high content of polyphenols, low pH value, and elevated salt concentration (Belligno et al., 2005; Doula et al., 2012; Ashraf et al., 2014; Khdair et al., 2019). Their unsustainable use can lead to healthy problems and environmental costs associated with illegal landfills and illegal management of wastes (export activities), as well as additional costs due to a non-realized circular economy market development (COWI, 2019). At the same time, orange and olive wastes, for their chemical composition, especially for the high level of nutritional components, could offer many opportunities of use.

* Corresponding author: Telephone number: 003909651694364, Fax number: 003909651694550
E-mail address: amuscolo@unirc.it (M. A).

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Orange residues contain water (75–85%), mono- and disaccharides (6–8%), and a limited level of oils in the peel waste (Wikandari et al. 2015; Moncada et al., 2016; Restrepo-serna et al., 2018). To valorize the orange wastes (OW), new technical solutions such as pectin extraction (Fakayode and Adobi, 2018), dietary fiber extraction (Sang et al., 2021), biogas production (Rokaya et al., 2019) and essential oil (particularly D-limonene) extraction (Siddiqui et al., 2021) are nowadays utilized to convert potential environmental hazards and economic issues (Palmeros Parada et al., 2017) into resources, however, the economic viability of these alternatives is not ensured because of the high-energy costs of these processes. Ortiz-Sánchez et al. (2021) evaluated from experimental, technical, and economic perspectives the production of essential oil, pectin, and biogas from OW and their results evidenced that the value-added products can be obtained in energetic and economic feasible way, mainly at low scales.

For the above-mentioned reasons, at present, only a small part of these biowastes is used to recover bioactive compounds for food, cosmetic and pharmaceutic industries, to produce green energy and animal feed, while the majority of these wastes are delivered to landfills yet.

Olive mainly used to produce oil, moreover in Mediterranean countries, generates enormous quantity of wastes not only wood, branches, leaves but also by-products (olive pomace, olive mill wastewater, olive stones) with negative environmental impact and high costs for management and disposal (Galanakis, 2017).Olive wastes and by-products rich in nutrients phenols, hemicelluloses, fat and proteins, were in the past spread on the land, but now much researches have been done to develop newer and higher biotechnological pathways to economically valorize the byproducts, by using thermochemical (via pelleting or pyrolysis, for heat and electricity) or (bio)chemical (fractionation, extraction, anaerobic digestion, for e.g., bioethanol, biophenols, biofertilizers, biogas) processes (Negro et al., 2017). New tendency consists of using olive by-products as food additives or nutraceuticals in the food and pharmaceutical industries, but the conversion technologies and the new proposed waste valorization methods are not yet economically feasible and implemented at an industrial scale.

The competitiveness of orange and olive processing industries, that generally spend a lot of their annual budget to residue treatment, can become feasible if environmentally and economically waste system management that combines efficient low-cost technology for their treatment and valorization will be adopted. Among the different waste-valorization techniques, composting is an easy, cheap biological process that can be used to convert recalcitrant biomass as orange and olive wastes into humus like substances under controlled optimum environmental conditions. Canet et al. (2008), during 9-10 month of olive waste composting (olive waste 50% and animal manures 50%), observed a decrease in organic matter and an increase in the concentrations of nutrient and humic substances, together with large increases in pH and salinity, the latter represent a great disadvantage for agronomy. In a previous research, Muscolo et al. (2018), using olive pomace at 90% highlighted that compost maturity can be mainly linked to composting setup parameters, rather than to raw material composition. Gelsomino et al. (2010) showed that after 5-month of aerobic bioconversion, orange waste reached an acceptable degree of maturity but the addition of orange compost to the soil selectively increased pH and electrical conductivity (EC) with negative consequence on plant growth. Other authors using different typologies of organic waste-derived fertilizers showed that the addition to soil, improved soil fertility, crop and residue yields in respect to the characteristics of the fertilizer used (Rigane et al., 2011; Beeby et al., 2020; Bhunia et al., 2021).

Muscolo et al. (2019) using three different methods to transform olive pomace (coming from a two-phase olive extraction plants) into fertilizers, evidenced that the efficiency of the fertilizer produced depended mainly on its chemical properties.

Based on the above statements, in this work we have used the three different waste transformation processes (aerobic, anaerobic digestions and crude waste pelleting) with the idea that is the intricacy of the method to determine the quality of the organic fertilizer output. The novelty of this study is to use two new recalcitrant agricultural wastes, that differ for their chemical characteristics olive pomace, coming from the three-phase olive extraction plants and orange waste (pastazzo) coming from citrus transforming industry to 1) verify if the three methods were equally and universally applicable to different biomasses; 2) check if there was a biomass -method specificity 3) assess if the efficacy/efficiency of the fertilizers produced on soil properties, growth and antioxidant properties of garlic (*allium sativum*) depended on the processes or on the own chemical characteristics.

The aim is to use the results of this study to provide company and other stakeholders with different solutions, economically and environmentally sustainable, to manage, selectively and on the basis of their potentiality the feedstock to increase the economy and the competitiveness of the agricultural sectors.

2. Materials and Methods

2.1. Olive Pomace and Orange Waste Chemical Characterization

The chemical properties of olive pomace (traditional three phases olive oil extraction process) and orange residue (pastazzo) have been detected following the methods reported in Muscolo et al. (2017). Heavy metals have been measured by using a Shimadzu model AA-680 atomic absorption spectrometer (Japan) (Pourjavid et al., 2014).

2.2. Aerobic digestion

Two separate processes to compost olive pomace and pastazzo were performed in bins, in triplicates (Muscolo et al., 2018, 2019). 1) 90% of pulp and kernel of olives and 10% of straw 2) 80% orange waste and 20% of straw were used. The choice of 90% for olive pomace and 80% for orange wastes respond to the exigence of using wastes as much as you can to verify their compostability and timing. The composting parameters used to compost both the agricultural wastes are indicated in Muscolo et al. (2018, 2019). In short, the composting parameters were setup as follow: a mesophilic temperature phase for 8 days at 29°C, a thermophilic temperature phase for 20 days at 50°C and a mesophilic temperature phase for 92 days at 27°C. The moisture was maintained at 50% and the oxygen percentage was >15%. The composting process took 4 months to obtain stable organic mixture. The time of composting was set up on the basis of results of previous experiments. Compost was air desiccated, sieved at 2 mm and homogenized.

2.3. Crude agricultural waste management

Pellets of 3/4 mm diam. were made by Steel Belt System s.r.l. as described in Muscolo et al. (2017a, 2019). The mixtures formed by 85% of liquid sulfur, 10% of bentonite clay (as support and carrier), and of 5% olive pomace or 5% pastazzo were pastilled in a special belt system.

2.4. Anaerobic digestion

Biogas energy plants (998 kWel), were in charge of Fattoria della piana s.r.l. The digester (U) 4240m^3 was loaded with olive waste 50%, animal manure and maize silage (50%) The digester (O) 3260m^3 was filled with orange waste 50%, and animal manure and maize silage (50%) (Panuccio et al., 2016; Muscolo et al., 2017b). In short, Biogas plant operators have selected process temperatures and retention times which are appropriate for the feedstock that had to be digested. Digester O: Process temperature: 40°C, pH 7.8, total volume of the digester: 7500 m^3, total volume loaded per day: 120 m^3/day, hydraulic retention time (HRT): 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C. Digester U: process temperature: 40°C, pH 8.0, total volume of the digester: 7420 m^3 total volume loaded per day: 120 m^3/day, hydraulic retention time (HRT) 60 days, minimum guaranteed retention time (MGRT) 16 h at 40°C. The digestates obtained were chemically characterized as reported in Muscolo et al. (2017b, 2019).

Composts, digestates and sulfur-based pads were analyzed as reported in Panuccio et al. (2016) and Muscolo et al. (2017a, 2019). The chemical characteristics of digestate, compost and sulfur-based pads with olive pomace or pastazzo are shown in Tables 3-4.

2.5. Soil analysis

Potted soils before treatment (CTR0) and six months after the different treatments were analyzed for physical and biological properties. Soil from Motta San Giovanni, Loc. Liso, Italy (LAT:38°0′15″12 N; LONG: 15°41′45″24 E) has a sandy-loam texture (11.85% clay, 23.21% silt, and 64.94% sand) as stated by FAO soil classification system (FAO, 1999). Moisture content were obtained drying the soil at 105°C (AOAC, 2005); pH and electric conductibility were detected in distilled water (AOAC, 2005); organic carbon was determined with Walkley–Black procedure (1934), and transformed into organic matter multiplying by 1.72; total nitrogen was assessed with Kjeldahl method (1883). Carbon content of humic and fulvic acids (Bettany et al. 1980) was assayed by Nelson and Sommers (1982) method. Water-soluble phenols were measured following the Box method (1983). Cationic exchange capacity (CEC) was determined with Mehlich methodology (1953). Fluorescein diacetate hydrolysis (FDA) reaction was determined as reported in Adam and Duncan (2001). Microbial biomass C (MBC) was detected in fresh samples (equivalent to 20 g DW) (Vance et al., 1987). Soluble organic C in the fumigated and unfumigated soil extracts were detected with the Walkley and Black method (1934). Soluble carbon was converted into biomass by using an extraction efficiency coefficient of 0.38 (Vance et al., 1987). Dehydrogenase (DH) activity was detected according von Mersi and Schinner (1991) method.

2.6. Plant material

Garlic plants were grown for six months in pots (30 cm diameter) filled with 9 kg of sandy-loam soil with the addition of: 160 g composted pastazzo or olive pomace, Pots were amended with S-bentonite + orange residue (SBOr); S-bentonite + olive pomace (SBOp) pads at the concentration of 1.4 g corresponding to 476 kg S ha^{-1} dose generally used in agriculture to lower the pH and to replenish S (Severson and Shacklette, 1988; Muscolo et al., 2017). 50% (w/w) of orange or olive digestate. Not amended soil was used as control. The quantity of compost, sulfur-based pads and digestate (C) have been selected on the basis of previous in vitro and in field results showing their better efficiency on soil and plant. (data not shown). The experimental design consisted of six pots for each treatment. The experiment was conducted in glass house to protect soil from rainfall, managing the irrigation system to maintain 70% of field capacity at a temperature of 25°C. After six months from sowing, the measured growth parameters of garlic plants were: leaf number and length (cm), root length (cm), bulb diameter (cm).

2.7. Preparation of garlic extracts

The extracts prepared as described by Kang (2015) were modified as reported in Muscolo et al. (2020). All extracts have been executed in triplicate.

2.8. Determination of total phenolic compounds and total flavonoids

Total phenols were measured using the Folin-Ciocalteu assay with a few changes (Muscolo et al., 2020). Sample absorbance were measured at 760 nm. Phenol content was expressed as mg gallic acid/100 g FW on the basis of a standard curve obtained with gallic acid (0-200 mg/L).

Flavonoids were tested with the colorimetric method of Djeridane et al. (2006), and expressed as rutin (R) E/g FW on the basis of a calibration curve obtained with rutin (Muscolo et al., 2020).

2.9. Antioxidant activity detection

DPPH• scavenging assay was assayed as reported in Papalia et al. (2017). DPPH activity was expressed as μM of Trolox (T) equivalents (E) using a calibration curve (1.0 to 50 μM T).

The ABTS assay (TE antioxidant capacity assay TEAC) was in accord with Re et al. (1999) method. Sample absorbance was measured at 734 nm. TEAC activity was expressed as μM Trolox (T) equivalents (E) using a reference curve (in the range from 1.0 to 50 μM) of T.

The oxygen radical absorbance capacity (ORAC) assay was carried out as in Muscolo et al. (2020). ORAC values were expressed as μmol TE mg^{-1} FW using a Trolox (10–100 μmol L^{-1}) calibration curve. All reagents were purchased from Sigma Aldrich Chemical Co.

2.10. Statistical analysis

Data are expressed as means ± standard error. Statistical analyses were processed using one-way analysis of variance (ANOVA) and means were compared with the Tukey's test ($P < 0.05$). Two-way ANOVA was used to test the effects of the factors (byproducts and wastes) on selected soil properties and on the antioxidant activity and bio-compounds of garlic. Data were processed with SYSTAT 13.0 for Windows (SPSS Inc.).

3. Results and Discussion

3.1. Waste and fertilizer chemical characteristics

The chemical characterization of these two wastes (Table 1) evidenced substantial and significant differences ($p \leq 0.05$) showing that olive pomace contained significantly more carbon, nitrogen and total phenols, conversely pastazzo contained a greater amount of nutrients and less amount of sodium and chloride. Electric conductivity and pH were not significantly different between the two agricultural wastes (Table 1). The content of heavy metals as Pb, Ni, Cd, Cr, and Co (Table 2) were low or negligible, so the use of both wastes for the production of fertilizers would not constitute an environmental and a healthy risk due to the accumulation of these elements in soils and plants. The chemical properties of both agricultural wastes fall in any case within the ranges commonly reported in literature for these materials (Mari et al., 2003; Doymaz et al., 2004; Manios et al., 2004). Currently in Italy in the biogas process, despite the high content of nutrients and carbon, only a small part of these wastes is recycled (Hollins et al., 2017), mostly instead are uncontrollably disposed on agricultural land with environmental damage due to uncontrolled fermentations that can lead to the production of

Table 1
Chemical properties of olive pomace and orange residue. The data are the mean of three replicates ± standard error (n=6). Different letters in the same row indicate, significant differences (Tukey's test, *$p \leq 0.05$).

Chemical properties	Olive pomace	Orange residue
pH	$5.03^a \pm 0.1$	$5.16^a \pm 0.2$
E.C (mS/cm)	$12.00^a \pm 1.1$	$10.00^a \pm 0.9$
Moisture (%)	$86.70^a \pm 3.2$	$83.60^a \pm 2.9$
C (%)	$59.62^a \pm 1.9$	$48.62^b \pm 2.5$
Total N (%)	$1.29^b \pm 0.2$	$2.00^a \pm 0.3$
C/N	$29.81^b \pm 1.9$	$37.7^a \pm 1.7$
Na^+ (mg g^{-1} dw)	$1.95^a \pm 0.5$	$0.97^b \pm 0.2$
NH_4^+ (mg g^{-1} dw)	$0.23^b \pm 0.03$	$0.33^a \pm 0.04$
K^+(mg g^{-1} dw)	$38.22^b \pm 2.3$	$49.22^a \pm 2.6$
Mg^{2+} (mg g^{-1} dw)	$2.03^b \pm 0.4$	$4.23^a \pm 0.7$
Ca^{2+} (mg g^{-1} dw)	$2.33^b \pm 0.7$	$9.33^a \pm 1.0$
Cl^- (mg g^{-1} dw)	$3.73^a \pm 0.5$	$2.44^b \pm 0.6$
PO_4^{3-}(mg g^{-1} dw)	$2.00^a \pm 0.4$	$1.09^b \pm 0.3$
SO_4^{2-}(mg g^{-1} dw)	nd	nd
Water soluble phenols (mg TAE g^{-1} dw)	$1.80^a \pm 0.4$	$0.53^b \pm 0.2$

Table 2
Heavy metals (mg/kg) in olive pomace and orange residue. The data are the mean of three replicates ± standard error (n=6). Different letters in the same row indicate, significant differences (Tukey's test, *p ≤ 0.05).

Heavy metals	Olive pomace	Orange residue
Cadmium	4.0^a x10^{-4}	4.5^b x10^{-6}
Lead	6.0^a x10^{-3}	5.0^b x10^{-5}
Zinc	2.2^a x10^{-2}	4.2^b x10^{-5}
Nichel	1.0^a x10^{-3}	2.0^b x10^{-4}
Mercury	7.0^a x10^{-3}	2.0^b x10^{-5}
Copper	nd	4.7×10^{-5}
Chromium	nd	$< 5 \times 10^{-8}$

toxic intermediate chemical compounds. (Doula et al., 2012). Slorach et al. (2019) evidenced that the application of digestate to land with the release of ammonia and nitrate leaded to higher marine eutrophication, terrestrial acidification and particulate matter formation (Agapiou et al., 2016). Satari et al. (2018) highlighted the importance to valorize citrus wastes to reduce their negative environmental impacts and, to achieve a circular bio economy creating additional profit.

The chemical analysis evidenced different chemical characteristics among compost, digestate and sulfur-based pads produced from both wastes, (Tables 3 and 4). pH was mostly alkaline in digestate. EC was low and similar in all the byproducts analyzed. Organic carbon content was significantly higher in compost and digestate than sulfur-based pads. Total nitrogen was more abundant in the digestate from olive (5.7%) and orange (5.2%) wastes followed by compost from olive (2.5%) and orange (2.7%) wastes and pads with olive (0.2%) and orange (0.9%) wastes. C/N ratio was much higher compost (18.1) than digestate and pads particularly in those coming from orange waste (Table 3). Nutrients were more concentrated in compost than in the other byproducts and mainly in compost from orange waste. The greatest amount of water-soluble phenols was in the digestate and much more in that produced by olive pomace. No significant differences in ON/TN ratio have been observed among the three byproducts coming from the transformation processes of both wastes. NH_4^+-N/NO_3^-N ratio was in absolute the highest in all the byproducts coming from the transformation process of olive pomace (Table 4). It was the lowest in the digestates and the highest in sulfur-based pads coming from both biomasses.

Table 3
Chemical characteristics of compost, digestate, and pads coming from olive wastes. The data are the mean of three replicates ± standard deviation(n=9). Different letters in the same row indicate significant differences (Tukey's test, *p ≤ 0.05).

Chemical characteristics	Compost	Digestate	Pad
pH	6.3^{b*}±0.05	8.5^a±0.20	6.4^b±0.18
Bulk Density (Kg/m^3)	598^b±9.0	788^a±8.2	nd
E.C (mS/cm)	1.3^a±0.25	1.3^a±0.20	1.0^a±0.10
Moisture (%)	47^b±3.2	64^a±7.1	nd
C (%)	44^a±2.40	45^a±1.40	2.5^b±0.14
Total N (%)	2.5^b±0.22	5.7^a±0.20	0.2^c±0.03
C/N	17.6^a±1.6	7.9^c±0.5	12.5^b±1.4
Na^+ (mg g^{-1} dw)	1.0^a±0.06	0.9^a±0.08	0.16^b±0.04
NH_4^+ (mg g^{-1} dw)	0.08^a±0.02	0.04^a±0.02	0.06^a±0.10
K^+(mg g^{-1} dw)	17^a±1.50	0.58^b±0.02	0.39^c±0.04
Mg^{2+} (mg g^{-1} dw)	1.40^a±0.06	0.58^b±0.08	0.49^b±0.03
Ca^{2+} (mg g^{-1} dw)	2.5^a±0.3	1.6^b±0.2	0.13^c±0.01
Cl^- (mg g^{-1} dw)	nd	0.68^a±0.07	0.18^b±0.01
NO_2^-(mg g^{-1} dw)	nd	nd	nd
NO_3^-(mg g^{-1} dw)	0.10^b±0.002	0.41^a±0.03	0.10^b±0.0001
PO_4^{3-}(mg g^{-1} dw)	0.43^a±0.03	0.47^a±0.06	0.13^b±0.02
SO_4^{2-}(mg g^{-1} dw)	0.27±0.02	nd	nd
S (%)	nd	nd	85±6
Water soluble phenols (mg TAE g^{-1} d.w)	2.44^b±0.06	5.24^a±1	1.23^c±0.13
ON/TN	93^a±5	92^a±8	70^b±3
NH_4^+-N/NO_3^--N	2.81^a±0.13	0.34^c±0.07	2.14^b±0.11

Table 4
Chemical characteristics of compost, digestate, and pads coming from orange wastes. The data are the mean of three replicates ± standard deviation (n=9). Different letters, in the same row indicate, significant differences (Tukey's test, *p ≤ 0.05).

Chemical characteristics	Compost	Digestate	Pad
pH	7.6^{b*}±0.5	8.3^a±0.8	6.8^c±0.18
Bulk Density (Kg/m^3)	558^b±12	758^a±11	nd
E.C (mS/cm)	1.8^a±0.2	1.5^a±0.4	1.3^a±0.10
Moisture (%)	44^b±3	69^a±7	nd
C (%)	49^a±2.4	47^a±1.4	2.8^b±0.14
Total N (%)	2.7^b±0.8	5.2^a±0.9	0.9^c±0.03
C/N	18.1^a±1.6	9^b±0.9	9.3^b±1.4
Na^+ (mg g^{-1} dw)	1.0^a±0.2	0.8^a±0.1	0.12^a±0.04
NH_4^+ (mg g^{-1} dw)	0.03^b±0.01	0.03^b±0.01	0.09^a±0.01
K^+(mg g^{-1} dw)	18^a±1.3	3.58^b±0.5	1.32^c±0.04
Mg^{2+} (mg g^{-1} dw)	1.80^a±0.05	0.89^c±0.06	1.41^b±0.02
Ca^{2+} (mg g^{-1} dw)	2.9^a±0.2	1.8^b±0.1	1.1^c±0.01
Cl^- (mg g^{-1} dw)	nd	0.48^a±0.05	0.11^b±0.02
NO_2^-(mg g^{-1} dw)	nd	nd	nd
NO_3^-(mg g^{-1} dw)	0.1^b±0.01	0.32^a±0.02	0.2^b±0.0001
PO_4^{3-}(mg g^{-1} dw)	0.90^a±0.03	0.63^b±0.04	0.3^c±0.02
SO_4^{2-}(mg g^{-1} dw)	0.87±0.02	nd	nd
S (%)	nd	nd	85±6
Water soluble phenols (mg TAE g^{-1} dw)	1.3^b±0.6	2.13^a±0.5	1.0^b±0.13
ON/TN	99^a±3	93^a±5	67^b±3
NH_4^+-N/NO_3^--N	1.05^b±0.13	0.31^c±0.05	1.55^a±0.2

3.2. Fertilizer efficacy on soil

When added to soil, all treatments influenced positively the soil chemical properties compared to control, even if the effects were different and depended on the type of byproduct used and also on the starting organic wastes from which the byproducts came from. Regardless of the type of initial organic waste, the compost was the byproduct with the best effects on soil. Compost coming from orange waste was in absolute the most effective (Tables 5 and 6). Compost influenced more soil characteristics, enhancing significantly and concomitantly the amount of organic matter, nitrogen, CEC, hydrolytic and oxidative soil activities as well as MBC (Tables 5, 6). In soils treated with compost, the humification process predominated as explained by the greater value of HC compared to FC value. The increase of key soil properties due to compost addition can be related to the composition of compost itself. Compost contained more organic nitrogen, ammonium, and nutrients compared to the other byproducts, and even if the content of organic matter can be similar to that of digestate the compost contains more stable organic matter as indicated by C/N ratio and this is the reason of organic matter increase in treated soils. Additionally, when an organic matter is added to soil, microorganisms use it as energy source hence, only the less degradable fraction of OM that remains and contributes to soil organic matter. This stable fraction of the original OM is referred as the "effective organic matter" (EOM) (Veeken et al. 2017). The EOM, calculated as OM/HC ratio was the highest in soil amended with both composts and mainly with orange compost. Organic carbon and nitrogen content were significantly lower in soil treated with both typologies of sulfur-based pads than the other treatments, conversely MBC was the highest. WSP considerably enhanced in all the treatments and the highest amount was in soil treated with both digestates (Tables 5, 6).

The results evidenced that the type of byproducts, regardless of the type of waste, mostly influenced soil characteristics, while the effects due to the interaction of the two factors (byproduct × waste) were less significant (Table 7). In short, both the two types of wastes and the three recycling processes examined seemed to produce byproducts with promising fertilizing capabilities. The ranking of treatments to ameliorate soil fertility were as follow: compost, sulfur-based pads and digestate. These results evidenced a specificity between treatment and soil properties, pointing out as the effects of the single byproduct depended

Table 5
Physical and chemical properties of potted alkaline sandy-loam soils CTR0, and six months after the addtion of: composted olive pomace "A"; sulphur-bentonite + olive pomace, "B"; olive digestate "C". Not amended soil was used as control (CTR). The data are the mean of six replicates ± standard deviation (n=18). Different letters in the same row indicate, significant differences among the treatments (Tukey's test, $p \leq 0.05$).

	CTR0	CTR	A	B	C
Texture	SL	SL	SL	SL	SL
pH	8.5 ^{a} ±0.60	8.5 ^{a} ±0.60	7.5 ^{a} ±0.80	7.2 ^{a} ±0.40	8.00 ^{a} ±0.20
EC µS/cm	339 ^{c} ±11	350 ^{c} ±14	450 ^{a} ±10	419 ^{b} ±12	466 ^{a} ±8.00
WC %	21.0 ^{b} ±2.6	21.5 ^{b} ±2.81	27.4 ^{a} ±0.79	24.2 ^{ab} ±1.70	28.8 ^{a} ±1.76
WSP µg TAE g^{-1} d.s.	19 ^{c} ±2.10	14 ^{c} ±2.80	45 ^{b} ±1.60	41 ^{b} ±3.26	96 ^{a} ±4.00
TOC %	0.95 ^{b} ±0.16	0.9^{c} ±0.16	1.73 ^{a} ±0.15	1.3 ^{b} ±0.25	1.5 ^{b} ±0.30
TN %	0.15±0.01^{bc}	0.15±0.01^{bc}	0.30±0.02^{b}	0.21±0.04^{a}	0.18±0.03^{c}
C/N	6 ^{b} ±0.3	6 ^{b} ±0.3	5.8 ^{b} ±1	6.2 ^{b} ±0.5	8.3 ^{a} ±0.6
SOM %	1.63 ^{c} ±0.3	1.55 ^{c} ±0.27	2.97 ^{a} ±0.26	2.24 ^{b} ±0.13	2.58 ^{a} ±0.38
FDA µg fluorescein g^{-1} d.s.	42 ^{a} ±2	42 ^{a} ±2	47 ^{a} ±3	38 ^{b} ±2	40 ^{b} ±1
DH µg INTF g^{-1} d.s. h^{-1}	56 ^{c} ±2	57 ^{b} ±2.81	65 ^{a} ±1.86	48 ^{c} ±3.17	62 ^{ab} ±1.95
MBC µg C g^{-1} f.s.	813 ^{d} ±18	835 ^{c} ±18	1007 ^{b} ±21	1081 ^{a} ±44	861 ^{c} ±27
HC %	0.60 ^{a} ±0.05	0.60 ^{a} ±0.05	0.43 ^{b} ±0.02	0.66 ^{a} ±0.01	0.62 ^{a} ±0.03
FC %	0.40 ^{b} ±0.08	0.45 ^{b} ±0.08	0.26 ^{c} ±0.05	0.62 ^{a} ±0.03	0.60 ^{a} ±0.03
HC/FC	1.5^{b}±0.12	1.33^{b}±0.12	1.65^{a}±0.10	1.06^{c}±0.04	1.03^{c}±0.06
CSC cmol($^{+}$) Kg^{-1}	18.9 ^{b} ±1.6	18.7 ^{b} ±1.42	23.10 ^{a} ±1.58	22.3 ^{ab} ±1.23	23.4 ^{a} ±1.36

Table 6
Physical and chemical properties of potted alkaline sandy-loam soils CTR 0, and six months after the addition of: composted orange wastes "A"; sulphur-bentonite + orange wastes, "B"; orange digestate "C". Not amended soil was used as internal control (CTR). The data are the mean of six replicates ± standard deviation (n=18). Different letters in the same row indicate, significant differences among the treatments (Tukey's test, $p \leq 0.05$).

	CTR0	CTR	A	B	C
Texture	SL	SL	SL	SL	SL
pH	8.5 ^{a} ±0.60	8.5 ^{a} ±0.60	8.0 ^{a} ±0.80	7.2 ^{a} ±0.40	7.5 ^{a} ±0.20
EC µS/cm	339 ^{c} ±11	350 ^{c} ±14	410 ^{a} ±10	437 ^{b} ±12	479 ^{a} ±8.00
WC %	21.0 ^{b} ±2.6	21.5 ^{b} ±2.81	29.4 ^{a} ±0.79	21.2 ^{ab} ±1.70	26.8 ^{a} ±1.76
WSP µg TAE g^{-1} d.s	19 ^{c} ±2.10	14 ^{d} ±2.80	40 ^{b} ±1.60	39 ^{b} ±3.26	90 ^{a} ±4.00
TOC %	0.95 ^{b} ±0.16	0.9 ^{b} ±0.16	2.1 ^{a} ±0.15	1.5 ^{b} ±0.25	1.8 ^{a} ±0.30
TN %	0.15±0.01^{bc}	0.15±0.01^{bc}	0.33±0.02^{b}	0.25±0.04^{a}	0.19±0.03^{c}
C/N	6 ^{b} ±0.3	6 ^{b} ±0.3	6.3 ^{b} ±0.4	6 ^{b} ±0.5	9.5 ^{a} ±0.6
SOM %	1.63 ^{c} ±0.3	1.53 ^{c} ±0.3	3.62 ^{a} ±0.3	2.58 ^{b} ±0.4	3.1 ^{a} ±0.8
FDA µg fluorescein g^{-1} d.s	42 ^{a} ±2	42 ^{a} ±2	46 ^{a} ±2	48^{a} ±1	43^{a} ±3
DH µg INTF g^{-1} d.s. h^{-1}	56 ^{c} ±2	57 ^{c} ±2	69 ^{a} ±2	62 ^{b} ±3	64 ^{b} ±1.5
MBC µg C g^{-1} f.s	813 ^{d} ±18	835 ^{d} ±18	1100 ^{b} ±21	1180 ^{a} ±34	890 ^{c}±27
HC %	0.60 ^{a} ±0.05	0.60 ^{a} ±0.05	0.44 ^{b} ±0.02	0.65 ^{a} ±0.01	0.60 ^{a} ±0.03
FC %	0.40 ^{b} ±0.08	0.45 ^{b} ±0.08	0.22 ^{c} ±0.05	0.60 ^{a} ±0.03	0.58 ^{a} ±0.03
HC/FC	1.5^{b}±0.12	1.33^{b}±0.12	2^{a}±0.10	1.08^{c}±0.04	1.03^{c}±0.06
CSC cmol($^{+}$) Kg^{-1}	18.9 ^{b} ±1.6	18.7 ^{b} ±1.6	25 ^{a} ±1.5	23 ^{ab} ±1.3	24 ^{a} ±1

Table 7
Two-way ANOVA to test the effects of the factors (byproducts and wastes) on WSP, SOM, FDA, DHA and MBC of potted alkaline sandy-loam soils. ***p<0.001; ** p<0.01: *p<0.05.

	WSP	SOM	FDA	DHA	MBC
R^2	0.995	0.906	0.802	0.899	0.955
F-ratios					
Byproducts	1005***	42***	10**	28***	102***
Wastes	16***	19***	13**	26***	22***
B × W	n.s.	n.s	7**	10**	n.s.

on its own chemical characteristics. The results, evidencing the positive effects of all these byproducts, agreed with previous works highlighting as organic components were able to improve soil fertility (Muscolo et al., 2019; Ye et al., 2020; Cortes' et al., 2020). The efficient transformation of these wastes into useful byproducts, is in line with the directive 2008/98/EC on wastes, mainly focused on sustainable and controlled disposal of wastes to prevent soil and groundwater pollution (Doula et al., 2012).

3.3. Fertilizer efficacy on garlic growth and metabolism

The effectiveness of compost, digestate and sulfur-based pads was also determined by testing the growth and antioxidant properties of garlic, a worldwide consumed crop, to close the loop of both quality and sustainability. Results evidenced that in garlic plants, cultivated with byproducts, the number and length of leaves increased in respect to control (Table 8). No significant differences were observed in root length between control and treatments. The garlic bulb diameter increased in

Table 8
Growth parameters of garlic grown for 6 months in alkaline sandy-loam soils not amended (control, CTR) or amended with: A composted orange wastes; sulphur-bentonite + orange wastes; orange digestate; B composted olive pomace, sulphur-bentonite + olive pomace, olive digestate. The data are the mean of six replicates ± standard deviation (n=18). Different letters in the same row indicate, significant differences among the treatments (Tukey's test, $p \leq 0.05$).

A				
Garlic	CTR	Compost	SB+OR	digestate
Leaf length (cm)	28±1^{b}	42±3^{a}	39±4^{a}	33±3^{a}
Root length (cm)	20±1^{a}	21±2^{a}	21±1^{a}	20±2^{a}
Bulb diameter (mm)	10±1^{b}	15±2^{a}	14±2^{a}	14±1^{a}
Leaves (number)	4±1^{a}	7±2^{a}	6±1^{a}	6±1^{a}
B				
Garlic	CTR	Compost	SB+OP	digestate
Leaf length (cm)	28±1^{b}	35±2^{a}	34±1^{a}	30±2^{b}
Root length (cm)	20±1^{a}	20±2^{a}	21±2^{a}	20±1^{a}
Bulb diameter (mm)	10±1^{b}	14±1^{a}	13±2^{a}	13±1^{a}
Leaves (number)	4±1^{a}	6±2^{a}	5±2^{a}	5±1^{a}

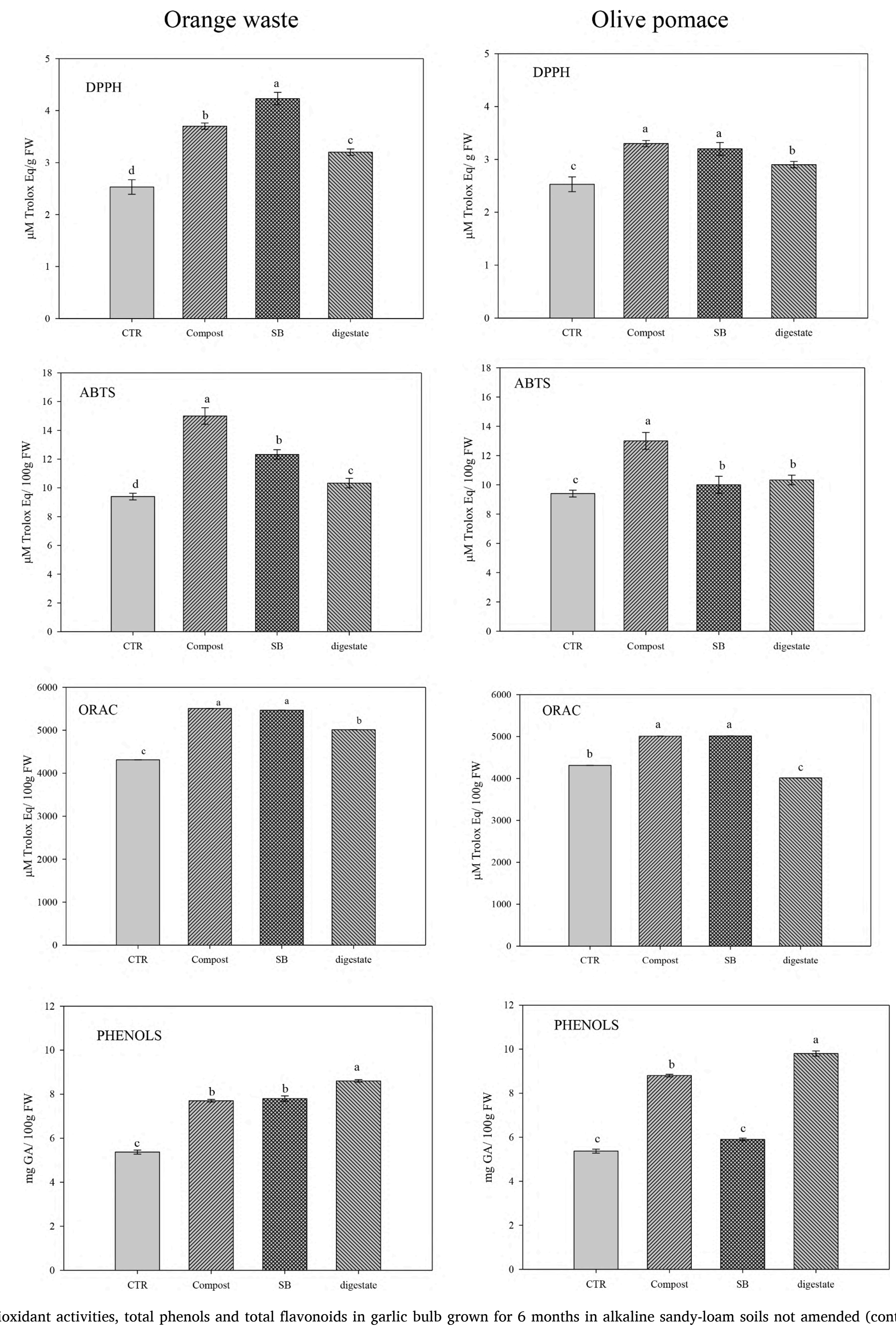

Fig. 1. Antioxidant activities, total phenols and total flavonoids in garlic bulb grown for 6 months in alkaline sandy-loam soils not amended (control, CTR) and amended with composted orange waste or olive pomace, sulfur-based pads or digestate. The data are the mean of six replicates ± standard deviation (n=18). Different letters indicate significant differences among the treatments (Tukey's test, $p \leq 0.05$). Two-way ANOVA was used to test the effects of the factors (byproducts and wastes) on antioxidants***$p<0.001$; ** $p<0.01$: *$p<0.05$.

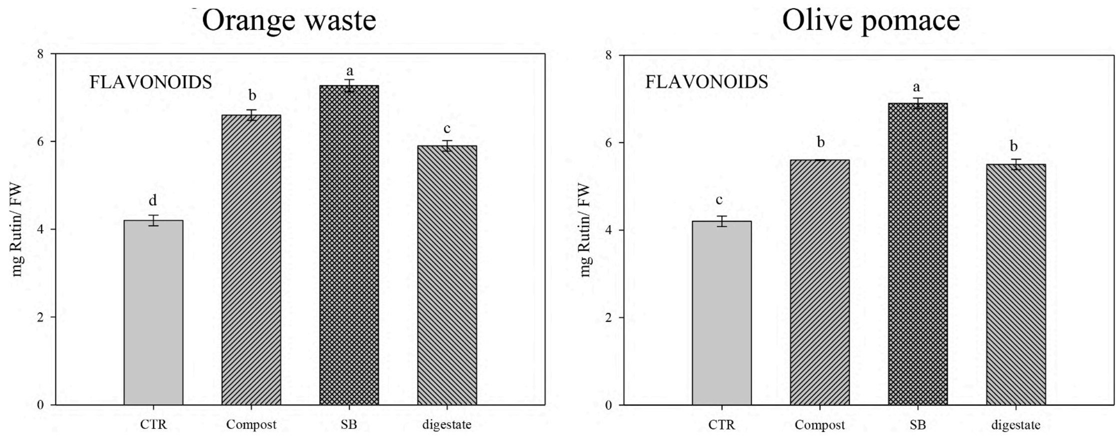

Fig. 1. (*continued*).

presence of compost, sulfur-based pads and digestate. The greatest increases were observed in presence of byproducts derived from orange waste (Table 8). These data evidenced a positive stimulatory effect of compost and sulfur-based pads. Antioxidant activities in terms of DPPH, ORAC and ABTS increased in presence of all treatments in respect to control, and the highest increment was observed with compost and sulfur-based pads coming from both orange waste and olive pomace (Fig. 1). Flavonoids increased significantly in presence of all byproducts in the following order: sulfur-based pad>compost>digestate>control. Total water-soluble phenols increased in presence of the treatments but with a different order of effectiveness: digestate>compost>sulfur-based pad>control (Fig. 1). These results evidenced as all the byproducts were able to increase the quality of garlic in terms of antioxidants, valuable inhibitors of free radical reactions, that protect cells against oxidative damage. Our results agree with previous studies reporting an increase in antioxidant compounds in broccoli (Naguib et al. 2012), sweet peppers (Del Amor et al. 2008), and tomatoes (Pieper and Barrett 2006) cultivated with bio-organic and organic fertilizers. The transformation of these agricultural wastes in resource using different processes lead to different final byproducts, all with positive effects on soil and plants. These byproducts can be used in an environmentally friendly agriculture and can positively affect the circular economy of the industrial sector and farms (Salomone et al., 2017). Each process has diverse set-up and is able to convert different amounts of wastes (90% or 80% for aerobic digestion, 50% for anaerobic digestion and 5% for sulfur-based pads) in different times (4 months aerobic digestion, 1 month for anaerobic digestion and 1 day for sulfur-based pads). The results show that each process, has a different competitivity from environmental, economic and/or agricultural standpoint. Composting (aerobic digestion process) has the greatest beneficial effects on soil and crops with low processing costs (no electricity and expenses of transport are necessary) though it has the longest production time. Anaerobic digestion is faster than aerobic digestion, produces energy or fuel, reducing the dependence of the agricultural sector on energy from fossil fuel, but it requires initial investments and conferral costs in the location of the plant. Sulfur-based pad production, despite the consumption of electricity, is a short processing cycle (one day) that disposes the crude agricultural wastes concomitantly with a great amount of another pollutant (Sulphur), reducing the emission of greenhouse gas in the atmosphere. In short, these results highlight that the different methodologies used such as recycling, valorization, and energy-producing processes, produce diverse types of products, all of high added-value that can be selectively chosen on the basis of specific farm and industry exigence to create an additional economical entry for the specific sectors.

4. Conclusion

Nowadays, converting agricultural wastes into byproducts is a priority that makes cleaner the environment, more fertile the soil, and improve farm bio economy. This study gives information on the processes that can be used to better convert these kinds of wastes into resource, evidencing as these different processes affect the chemical composition and in turn the potential added value of the end-products obtained. The recycling of these wastes can be considered a beneficial process leading to significant reductions in greenhouse gas emissions in the atmosphere for the elimination of a large amount of hazardous materials from the environment. Generally, orange and olive wastes release different amount of greenhouse gas; one ton of wet orange waste left on the ground emits 0.130 kg of CH_4, 30.900 kg of CO_2 and 0.069 kg of N_2O, while one ton of wet olive pomace produces, 1162.3 kg of CO_2, 122 kg of CH_4 and 0.12 kg of N_2O. In addition to organic wastes, Sulphur as residue of hydrocarbon refining processes generates hydrogen sulphide and Sulphur oxide causing environmental pollution, thus the use of high Sulphur percentage in the fertilizers can help to maintain a clean environment. Furthermore, the economic benefit of transforming the wastes in fertilizers will come not only from the sale of the new fertilizers produced but also by the money saved from the decrease in the production and use of chemical fertilizers and the reduction of costs for landfilling, Regarding the sustainability and profitability of compost process, the plants have to serve a local market, supplying their product within 50 km of the plant. This is equivalent at a cost of EUR 50–60 transport costs for 25 tons of biomass. The expenses are covered by end compost prices of around EUR 5/ton (EUR 125/for 25 tons). The environmental gain rises, in this case, from 60% decrease of CO_2 emission due to the reduction of landfill permanence of wastes and from the reduction in an average 9.7 tons of CO_2 equivalent emitted for every ton of mineral fertilizer less produced. Similarly, for the profitability of digestate, the plants have to be rationally distributed in the territory in order to receive short-range wastes. The production costs of digestate generally range from 10 to 30 Euro per ton for bio-waste treatment through anaerobic digestion. This value excludes the investment costs necessary for start-up, and costs are sensitive to technology used as well as input materials. In a context of high fossil fuel energy prices. the intrinsic value of the digestate can compensate the proportionately high price of fossil fertilizers quantified for nitrogen (34.5% ammonium nitrate) Euro 616 ton^{-1}, phosphate (46%)—Euro 525 ton^{-1} and • potassium (60% potassium chloride) —£534 ton^{-1}. On the basis of the above fertilizer costs, a ton of digestate was calculated to be worth a total of Euro 119,160. Sulfur-bentonite fertilizer advantage come from the selling of the pads that can be used mainly to recovery degraded lands and can be sell to 230/tons euros, to which must be added the euros saved by the reduction of CO_2, CH_4 and Sulphur emissions in the atmosphere.

In short, these results highlight that the different methodologies used such as recycling, valorization, and energy-producing processes, produce diverse types of products, all of high added-value that can be selectively chosen on the basis of specific farm and industry exigence. Results evidenced also new potential applications of these two kinds of wastes considering that the derived byproducts can be used not only to recovery soil fertility but also to improve the quality of crop species stimulating the synthesis of bio compounds with pharmaceutical and nutraceutical purposes. The production processes of these byproducts, whose benefits overcome risks, are valuable and greatly could create an additional economical entry for the agricultural sector.

Declaration of Competing Interests

Regarding the manuscript entitled "Recycling of agricultural (orange and olive) bio-wastes into ecofriendly fertilizers for improving soil and garlic quality." All the authors agree that there are no interests to declare.

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Chapter 3 - Effects of fertilizer produced from agro-industrial wastes on the quality of two different soils.

Marra Federica[1], Maffia Angela[1], Canino Francesco[1], Greco Carmelo[1], Mallamaci Carmelo[1] and Muscolo Adele[1].

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

Correspondence: amuscolo@unirc.it

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Effects of fertilizer produced from agro-industrial wastes on the quality of two different soils.

Abstract

A newly developed sustainable fertilizer, known as SB, was created by blending organic and mineral components using agro-industrial waste, Sulphur, and orange residue, bound together with bentonite. It was extensively tested on two distinct soils with different chemical and biological properties, comparing its effectiveness to traditional chemical (NPK) and organic (horse manure, HM) fertilizers, with unfertilized soil as a control (CTR). The introduction of SB did not alter soil texture but significantly impacted soil chemistry and biology. It positively influenced the labile fraction of soil organic matter, resulting in a 15% increase in soil microbial biomass, total phenolic content, cations, bacterial colonies, and enzyme activities, with varying effects depending on soil characteristics. In conclusion, SB represents a promising innovation for transitioning from traditional agriculture to a more sustainable and circular approach, offering economic and environmental benefits by reducing waste disposal costs and decreasing reliance on mineral fertilizers in line with circular economy principles. This study emphasizes the need to consider soil properties when optimizing fertilizer use

Keywords: Fertilizer production; soil enzyme activities; soil quality; soil fertility; sulphur-based fertilizer

1.Introduction

The global increase in population has led to a surge in worldwide food demand, significantly impacting soil due to intensive tillage and excessive fertilization practices. These practices, often irreversible, disrupt the delicate ecological balance of soil by affecting nutrient cycles, nutrient availability, and soil chemical properties. In addition to the pressing need to boost crop productivity, there is an equally urgent requirement to enhance the sustainability of the agricultural supply chain by reducing the reliance on agrochemicals and mineral fertilizers, aligning with the European Union's (EU) Green Deal program. Notably, this includes compliance with the Farm to Fork Strategy and the EU Biodiversity Strategy for 2030, both of which revolve around critical issues related to climate, the environment, and agriculture. These strategies envisage substantial reductions in pesticides, fertilizers, and antibiotics, accompanied by a significant upswing in organic farming. The overarching objective is to transition to a food chain grounded in circular bio-economy principles, reducing food waste and losses while embracing organic agriculture. The utilization of organic fertilizers to preserve soil fertility has long been a fundamental tenet of sustainable agriculture. Recently, there has been an increasing focus on organic fertilizer production from waste materials, aligning with the European Commission's goal of achieving a 30% reduction in non-renewable resource usage by recycling them into fertilizers. This concept of circularity underscores the repurposing of by-products, marking a shift from a fossil-based economy to a bioeconomy, with a paramount emphasis on nutrient recovery to mitigate the high energy costs associated with mineral fertilizer production processes. Crucially, the agri-food industry consistently generates organic wastes, with their quantity expected to reach 3.4 billion tonnes in the near future. If left to languish in landfills, these waste materials can give rise to significant local and global environmental issues. These include the emission of greenhouse gases, soil contamination, pollution of local water sources, and the eutrophication of riverbeds and freshwater reserves due to an excess of nitrogen. Incorporating these waste products into agricultural practices can play a pivotal role in recycling vital plant nutrients. However, it's important to note that the impact of these fertilizers on soil properties can vary widely based on the specific soil type, environmental conditions, and the type of fertilizer used, each exerting a different level of effectiveness in enhancing soil productivity. It's worth emphasizing that the use of chemical fertilizers is not advisable, as it can lead to several soil-related issues, such as soil compaction and degradation. Instead, a general recommendation is to increase the amount of soil organic matter (SOM) as an efficient means to enhance soil quality for sustainable agricultural production. SOM is widely acknowledged for its ability to improve soil quality and boost crop productivity. It achieves this by creating soil aggregates that enhance soil stability and by stimulating the activities of soil microorganisms. SOM also serves as a carbon source, which, through the mineralization process, results in an increased availability of essential nutrients for plant mineral nutrition. Furthermore, organic fertilization has garnered significant attention because its application promotes the biodiversity of soil bacteria. This microbial diversity not only drives secondary metabolic processes but also stimulates primary productivity (Shang et al. 2020). The reuse of waste materials for agricultural purposes, particularly citrus waste, has the potential to enhance soil quality by enriching soils with beneficial and effective microbes and nutrients (Corti et al. 2012). Microbial biomass plays a pivotal role in breaking down complex biomolecules into simpler forms, facilitating easier uptake by plants. Given the challenges posed by resource scarcity and waste disposal, the principles of the Circular Economy demand that waste management and the utilization of waste materials for sustainable raw material use be addressed comprehensively. Additionally, it's important to note that sulphur, the fourth most critical nutrient after nitrogen, tends to be deficient primarily in high-yield, arid, semiarid, and desertified soils (Yesmin et al. 2021). To enhance soil biodiversity and functionality through proper fertilization, the incorporation of sulfur into organic fertilizers derived from agricultural waste presents an avenue to bolster the soil's nutrient reservoirs while aligning with the principles of the circular economy, especially when utilizing reclaimed sulphur. Given its compatibility with other fertilizers and its suitability for early- stage and intensive plant growth, sulphur supplementation holds promise. Building upon these insights, the primary objective of this study was to assess the impact of a novel fertilizer composed of sulphur-bentonite and orange residue in open field conditions on different soils characterized by varying chemical and biochemical properties. This investigation encompassed varying concentrations of the fertilizer, with comparative evaluations against chemical fertilizer (NPK) and horse manure (HM). As a control, unfertilized soil (CTR) was also included. The central focus of this study revolved around the influence of soil characteristics on fertilizer effectiveness. Recognizing the paramount role soil attributes play in nutrient availability, pH balance, nutrient uptake, water retention, environmental consequences, and overall plant vitality, it is imperative to consider these factors when selecting a fertilizer. Neglecting soil characteristics during fertilizer selection can result in suboptimal nutrient utilization, impaired plant growth, and potential harm to the environment. Therefore, the investigation delved into the following aspects: 1) the fertilizer's impact on soil chemical properties; 2) the extent to which the new fertilizer affected soil quality, encompassing nutrients, soil enzymes, fungi, bacteria, and actinomyces; and 3) the influence of specific soil characteristics on the fertilizer's efficacy, all with the aim of elucidating changes in the quality and functionality of the two soils.

2. Materials and methods

2.1 Fertilizer production

Steel Belt System s.r.l. developed fertilizer in tablet of 3/4 mm as described in Muscolo et al. (2017, 2019). Sulphur was mixed with bentonite and orange rest of food industry (O). Elemental S was the principal component of fertilizer (Muscolo et al. 2020). The fertilizer was tested for pathogens (total coliforms, faecal coliforms, salmonella spp and Escherichia coli) and heavy metals to prevent unhealthy and dangerous effects on soil (Ben Said et al. 2017; Muscolo et al. 2021). Results evidenced absence of pathogens and heavy metals (Muscolo et al. 2021).

2.2 Soil treatment

The experiment was carried out in two soils differing for chemical and biological properties. A sandy- loam soil belonging to Cambisol (WRB, 2022) located in Motta San Giovanni, Loc. Liso, Italy (37.9991°N. 15.6999° E) arbitrarily named Motta, and a sandy clay loam soil belonging to Alisol (WRB, 2022) located in Lazzaro 37.9724° N. 15.6657° (arbitrarily named Lazzaro) were used for the experiments. Textural class of the two soils were identified using the Food and Agriculture Organization of the United Nations (FAO) soil classification system (FAO 2007). The soils were amended with four doses of the new fertilizer and precisely with 476 kg S ha^{-1} (SB, 1.4), 952 kg S ha^{-1} (SB, 2.8), 1428 kg S ha^{-1} (SB, 4.2) and 1904 kg S ha^{-1} (SB, 5.6). The different doses were chosen on the basis of literature data on the quantity of pure sulphur that is normally used in respect to soil texture, which range from 2200 kg S ha^{-1} to 3300 kg S ha^{-1} in sandy or clay soil, respectively (Severson and Shacklette 1988; Muscolo et al. 2017). Soil no fertilized was used as control (CTR), nitrogen: phosphorous: potassium (NPK, 20/10/10) as chemical fertilizer and horse manure (HM, 4.3 q/ha) as organic fertilizer. Soils were divided in plots of 1 m square each and fertilized. Each treatment was replicated six folds. The experiment was arranged in a randomized complete block design, the parcels were six 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.

2.3 Soil chemical analysis

Soil texture was detected following Bouyoucos (1962) method. Electric conductibility (EC) was tested in 1:5 soil/water suspension, after stirring at 15 rpm for 1 h. EC was detected by Hanna instrument conductivity meter; pH was determined in soil/solution ratio 1:2.5 with a glass electrode. Organic carbon was tested with Walkley and Black (1934) methodology. Total nitrogen (TN) was assessed with Kjeldahl method (1883). C/N was quantified as a carbon:nitrogen ratio. Water soluble phenols were extracted and analyzed as described by Kaminsky and Muller (1978) and monomeric and polyphenols were determined with Box (1983) method, using tannic acid as standard. The concen tration of water-soluble phenolic compounds was expressed as tannic acid equivalents (μg TAE g^{-1} D.W.). Cation Exchange Capacity was analysed with barium chloride method (Hendershot and Duquette 1986). Cations and anions were detected with ion chromatography (DIONEX ICS-1100), as described in Muscolo et al. (2022).

2.4 Soil biological analysis

For the detection of microbial biomass carbon (MBC) the chloroform fumigation-extraction procedure was used (Vance et al. 1987) on fresh soil. Fumigated and unfumigated soil sample extracts were used to detect soluble organic C (Walkley and Black 1934). To detect bacteria, fungi and actinomycetes 10 grams of each soil sample were extracted with 95 mL of 0.1% (w/v) solution of sodium pyrophosphate. Soil extract solutions were diluted (10^{-1} to 10^{-7}) and the shares were plated on agarized culture media, each specific for bacteria or fungi or actinomycetes (Elliot and Des Jardin 1999). Colony forming units (CFU) for each microorganism were counted as reported in Picci and Nannipieri (2003) and Eaton et al. (2005). Fluorescein diacetate hydrolase (FDA) activity was determined according to the method of Adam and Duncan (2001). Dehydrogenase (DHA) activity was assessed with Von Mersi and Schinner (1991) method. Catalase activity (CAT) was detected assessing the absorbance during the transformation of H_2O_2 to oxygen and water (Muscolo et al. 2017). 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. (2017). Urease activity was determined as reported in Kandeler and Gerber (1988) with few modifications described in Sidari et al. (2008). 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}. Beta-glucosidase activity was assessed following Eivazi and Tabatabai (1988) method and the results have been expressed as µg of para-nitrophenol (p-NP) g h^{-1}.

2.5 Statistical analysis

Analysis of variance was used for all the data sets. One-way ANOVA with Tukey's Honestly. Significant difference tests for analysing the effects of fertilizers on each of the parameters measured were used. ANOVA and T-test were done with SPSS software. The effects were significant at $p \leq 0.01$. To analyze the relationships among the different fertilizers and the soil parameters in the two different sites, Principal Component Analysis (PCA) was used.

3. Results and discussion

3.1 Soil chemical and biochemical characteristics of unamended soils

The selection of the two soils was deliberate, considering their distinct chemical and biological properties as detailed in Table 1. The Cambisol in Motta San Giovanni (CTR) exhibited a sandy-loam texture, comprising 65% sand, while the Allisol in Lazzaro (CTR) presented a sandy-clay-loam texture with 50% sand, 23% clay, and 27% silt. Notably, there were no significant disparities in pH and electrical conductivity between the two soils. In terms of organic content, Motta soil boasted a higher organic carbon content (1.98%) and total nitrogen content (0.20%), along with a greater cation exchange capacity compared to Lazzaro soil, which had 1.4%

organic carbon, 0.16% total nitrogen, and a lower cation exchange capacity. Conversely, Lazzaro soil exhibited a larger microbial biomass C (Table 1) and total phenol content. Furthermore, Lazzaro soil contained a more extensive array of anions and cations than Motta soil (Table 1). In relation to biological attributes, the activities of FDA and DHA displayed a similar trend in both soils, while catalase activity was notably higher in Motta soil (Table 1). The composition and dynamics of soil organic matter, especially the balance between its stable (humic substances) and labile components, have a direct correlation with nutrient release (Zanin et al. 2019) and, consequently, soil fertility and quality (Gerke 2022). Our data underscored that Lazzaro soil harbored a richer pool of nutrients, owing to a higher microbial biomass, as well as a greater abundance of bacteria and fungi (Table 1). These microorganisms play pivotal roles in nutrient cycling, aligning with previous studies by Prosser (2007) and Shay et al. (2015). These findings are further supported by the reduced quantity of organic matter in Lazzaro soil, attributed to the substantial mineralization driven by the numerous colonies of fungi and bacteria (Hicks et al. 2021), which are the primary producers of soil enzymes (Baćmaga et al. 2021). Soil microbial biomass and enzy- matic activities, particularly hydrolase activities, are intimately involved in organic matter turnover and nutrient cycling, making them sensitive indicators of soil fertility (Sekaran et al. 2021). Collectively, the data from biochemical and biological parameters underscore the disparities between the two soils, with Lazzaro soil emerging as the more fertile substrate. These variations in biochemical parameters served as a basis for investigating the impacts of different fertilization practices, encompassing organic, chemical, and organic-mineral amendments, on soil quality and health.

Table 1. Chemical and biochemical properties of soil before the experiment located in Motta and Lazzaro. Soil texture (percentage of sand, silt and clay); pH_{H2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (µS cm^{-1}); TP = total 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} soil); CEC= cation exchange capacity ($cmol^{(+)}$ kg^{-1}), dehydrogenase, (DHA), fluorescein diacetate hydrolase (FDA), Catalase (CAT).

	Motta	**Lazzaro**
SANDY	65 ± 10[a]	50 ± 1[a]
CLAY	12 ± 4[a]	23 ± 0.8[a]
SILT	23 ± 3[a]	27 ± 0.9[a]
TEXTURE	Sandy-loam	Sandy-clay-loam
PH (H2O)	8.4 ± 0.1[a]	8.4 ± 0.1[a]
PH (KCL)	6.91 ± 0.1[a]	6.96 ± 0.1[a]
EC	301 ± 10[a]	301 ± 8[a]
TP	280 ± 12[b]	327 ± 12[a]
MBC	845 ± 12[b]	1122 ± 22[a]
CEC	28 ± 1[a]	11 ± 2[b]
OC	1.98 ± 0.5[a]	1.4 ± 0.2[a]
TN	0.20 ± 0.02[a]	0.16 ± 0.02[a]
C/N	9.9 ± 1.5[a]	8.8 ± 1.3[a]
OM	3.4 ± 0.6[a]	2.4 ± 0.3[a]
ß-GLUCOSIDASE	514 ± 6[a]	208 ± 5[b]
PROTEASE	148 ± 7[b]	166 ± 5[a]
UREASE	350 ± 12[a]	253 ± 17[b]
FDA	10 ± 1[a]	10 ± 1[a]
DHA	5.3 ± 1[a]	5.2 ± 1[a]
CAT	3.7 ± 1[a]	1.2 ± 0.5[b]
BACTERIA COLONY	1 ± 0.07[b]	163 ± 17[a]
FUNGI COLONY	30 ± 1[a]	36 ± 2[b]
ACTINOMYCETES	48 ± 3[b]	65 ± 5[a]
CALCIUM	3.1 ± 0.3[a]	1.9 ± 0.1[b]
MAGNESIUM	1.8 ± 0.2[a]	2.2 ± 0.4[a]
POTASSIUM	1.2 ± 0.1[b]	4.0 ± 0.3[a]
AMMONIUM	15 ± 1[b]	50 ± 5[a]
SULPHATE	44 ± 2[a]	48 ± 2[a]

3.2 Soil characteristics 6 months after treatments with the different fertilizers

No changes in textural class was observed in soils six months after the treatments with the different fertilizers in both locations in respect to CTR (Tables 2 and 3). These findings confirm that texture is a soil property that remains relatively resistant to change. Fertilization, whether with organic or mineral fertilizers, primarily affects the distribution ratio of soil aggregates due to its influence on soil carbon dynamics (Niu et al. 2022). Notably, there was a decrease in pH only in the presence of Sulphur-based fertilizers at high concentrations, underscoring sulphur's role as an acidifying soil amendment (Mehdi et al. 2019). In Motta soil, this decrease in pH was observed only in water, not in KCl, indicating that the effects of sulphur-based fertilizers were more related to the acidification of the soil solution in circulation than to the reserve acidity in the colloids. Conversely, in Lazzaro soil, a reduction in pH values, both in water and KCl, was noted in soils treated with sulphur-based fertilizers (Table 3). These results can be correlated with some observed differences between the two soils, particularly the high level of organic matter detected in Motta soil (Table 2). This organic matter, as demonstrated by Dvořáčková et al. (2022), has the potential to act as a buffer against soil acidification due to its cation-binding capacity. This is further supported by the higher cation exchange capacity (CEC) observed in Motta soil due to its greater organic matter content (Table 2). These findings align with prior research by Solly et al. (2020), which established a correlation between CEC, organic matter content, and soil buffering capacity. The electrical conductivity (EC) in the untreated soils (CTR) was similar in both locations (Tables 2 and 3). In Motta, fertilization did not have a significant impact on EC. In contrast, in Lazzaro, EC decreased in the presence of sulphur-based fertilizers. Total phenols, precursors of humic substances that serve as a rich carbon source for microbial biomass and potent antioxidants (Min et al. 2015), increased in soils treated with sulphur- based fertilizers in comparison to other treatments in both locations (Tables 2 and 3). These findings align with the greater microbial biomass carbon (MBC) and higher C/N values observed in soils treated with sulphur-based fertilizers, indicating a distinct trend in organic matter dynamics between the two soils. Among the detected cations, magnesium and calcium were found in higher concentrations in soils fertilized with sulphur-bentonite compared to other fertilizer types in both locations (Figure 1a and b).

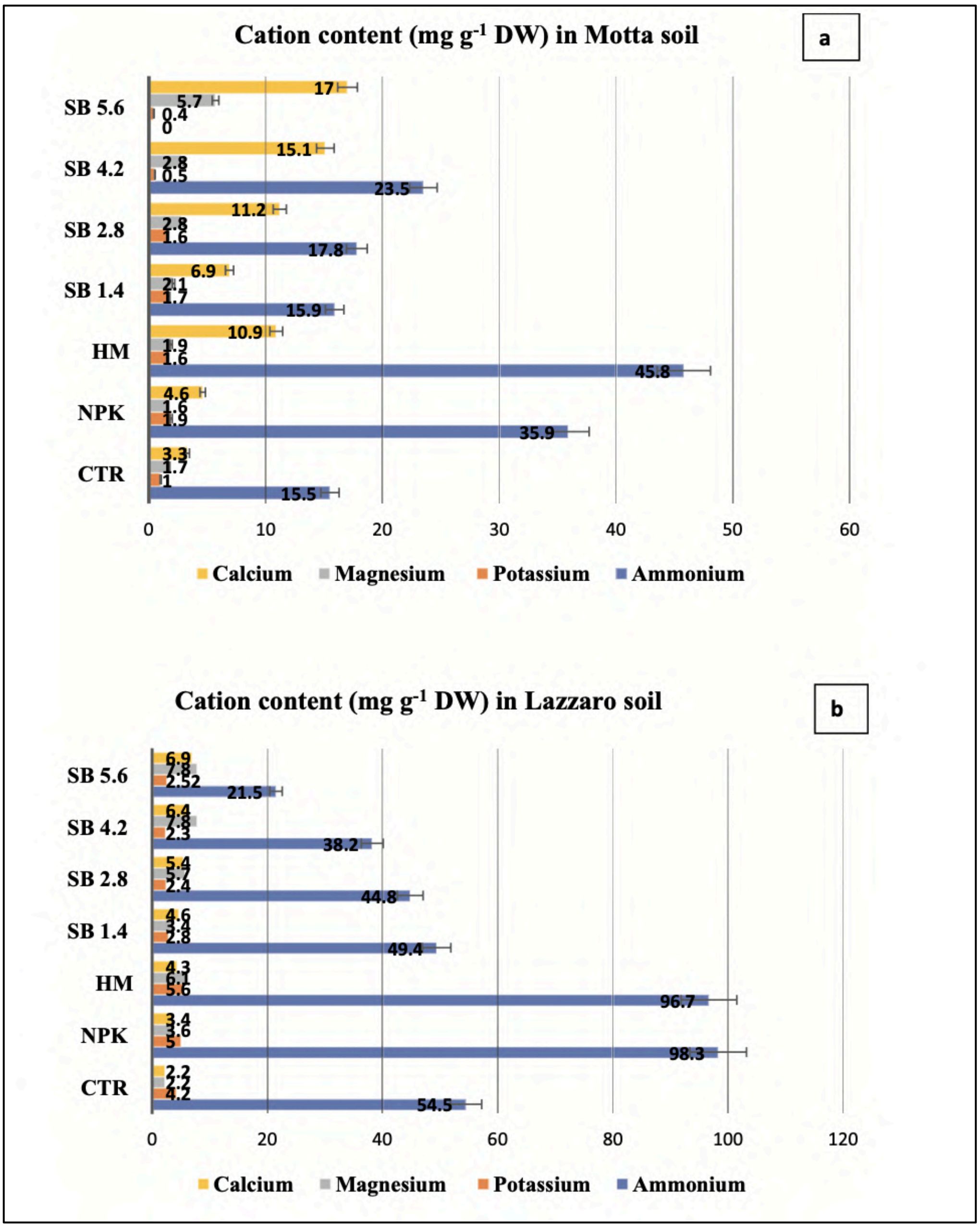

Figure 1 (a, b) Cation content in Motta San Giovanni (a) and Lazzaro (b) soils, 6 months after the amendment with NPK = nitrogen: phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6) and CTR = control, soil without fertilizer. Data are the means of six independent experiments and bars represent the standard error of the parameters analysed. Data are the means of three replicates (*n* = 18) ± standard errors**.**

The sulphur content in the soil may indirectly influence the uptake levels of other nutrients. Research has shown that sulphur has a positive effect on calcium, leading to an increase in total phosphorus and magnesium levels, while reducing potassium content (Skwierawska et al.

2016). The observed differences can be attributed to the CEC value, which was highest in the presence of sulphur- bentonite (SB), capable of retaining more cations, especially bivalent ones, compared to monovalent ions (Elbaalawy et al. 2023). Nitrate levels in the presence of sulphur bentonite were similar to those with horse manure (HM) and NPK, and they slightly increased with higher SB concentrations, while ammonium levels decreased, likely because monovalent ions were less retained by soil exchange sites compared to bivalent ions. Furthermore, the higher abundance of these cations may also result from the organic matter mineralization process, coupled with the formation of sulphate by soil microorganisms. Calcium and magnesium sulphate, in particular, play a role in retaining calcium and magnesium cations in the soil, preventing their precipitation at the prevailing soil pH, as corroborated by previous studies (Skwierawska et al. 2008). Among the anions, sulphate, malate, and phosphate were found in the greatest abundance in both Motta and Lazzaro soils treated with sulphur-based fertilizers (Figure 2a and b).

Table 2. Chemical and biochemical properties of soil located in Motta San Giovanni, 6 months after treatments with the different fertilizers. CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulfur bento- nite + orange residue. Soil texture (percentage of sand, silt and clay); pH_{H2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (μS cm^{-1}); TP = total 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} soil); CEC = cation exchange capacity ($cmol^{(+)}$ kg^{-1}).

Parameter	CTR	NPK	HM	SB 1.4	SB 2.8	SB 4.2	SB 5.6
Sandy	65 ± 10^{a}	65 ± 12^{a}	65 ± 11^{a}	65 ± 12^{a}	65 ± 9^{a}	65 ± 12^{a}	65 ± 11^{a}
Clay	12 ± 4^{a}	12 ± 2^{a}	12 ± 3^{a}	12 ± 2^{a}	12 ± 2^{a}	12 ± 4^{a}	12 ± 3^{a}
Silt	23 ± 3^{a}	23 ± 2^{a}	23 ± 4^{a}	23 ± 1^{a}	23 ± 2^{a}	23 ± 2^{a}	23 ± 3^{a}
Texture	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam
pH (H2O)	8.43 ± 0.1^{a}	8.47 ± 0.2^{a}	8.46 ± 0.1^{a}	8.41 ± 0.2^{a}	8.43 ± 0.1^{a}	8.12 ± 0.1^{b}	8.19 ± 0.1^{b}
pH (KCl)	6.94 ± 0.1^{a}	7.01 ± 0.1^{a}	6.99 ± 0.1^{a}	6.97 ± 0.2^{a}	7.04 ± 0.1^{a}	7.01 ± 0.2^{a}	7.03 ± 0.1^{a}
EC	302 ± 10^{a}	301 ± 8^{a}	297 ± 12^{a}	296 ± 10^{a}	302 ± 13^{a}	267 ± 12^{a}	278 ± 10^{a}
TP	282 ± 12^{b}	320 ± 10^{b}	315 ± 10^{b}	280 ± 10^{b}	332 ± 15^{ab}	352 ± 20^{a}	357 ± 20^{a}
MBC	835 ± 12^{c}	798 ± 15^{d}	997 ± 12^{a}	845 ± 15^{c}	912 ± 12^{b}	933 ± 14^{b}	923 ± 16^{b}
CEC	27.8 ± 1^{a}	28 ± 1.5^{a}	30 ± 0.8^{ab}	31 ± 12^{ab}	38 ± 2^{a}	36 ± 1^{a}	36 ± 1.4^{a}
OC	1.98 ± 0.5^{a}	1.69 ± 0.3^{a}	2.15 ± 0.4^{a}	1.92 ± 0.5^{a}	2.24 ± 0.3^{a}	2.24 ± 0.5^{a}	2.08 ± 0.5^{a}
TN	0.20 ± 0.02^{a}	.23 ± 0.02^{a}	0.21 ± 0.03^{a}	0.19 ± 0.01^{a}	0.15 ± 0.01^{b}	0.12 ± 0.02^{b}	0.10 ± 0.03^{b}
C/N	9.9 ± 1.5^{c}	7.39 ± 1.4^{d}	10.1 ± 1.3^{c}	10.2 ± 1.7^{c}	15.3 ± 1^{b}	20 ± 1.5^{a}	20.9 ± 1.5^{a}
OM	3.42 ± 0.6^{a}	2.91 ± 0.5^{a}	3.70 ± 0.5^{a}	3.30 ± 0.6^{a}	3.85 ± 0.5^{a}	3.85 ± 0.4^{a}	3.57 ± 0.3^{a}

Table 3. Chemical and biochemical properties of soil located in Lazzaro, 6 months after treatments with the different fertilizers. CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulfur bento- nite + orange residue. Soil texture (percentage of sand, silt and clay); pH_{H2O} in water and pH_{KCl} in potassium chloride; EC = electric conductivity (µS cm^{-1}); TP = total 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} soil); CEC = cation exchange capacity ($cmol^{(+)}$ kg^{-1}).

Parameter	CTR	NPK	HM	SB 1.4	SB 2.8	SB 4.2	SB 5.6
Sandy	50 ± 1^{a}	50 ± 2^{a}	50 ± 3^{a}	50 ± 4^{a}	50 ± 2^{a}	50 ± 3^{a}	50 ± 2^{a}
Clay	23 ± 0.8^{a}	23 ± 1^{a}	23 ± 0.9^{a}	23 ± 0.5^{a}	23 ± 0.8^{a}	23 ± 0.7^{a}	23 ± 0.6^{a}
Silt	27 ± 0.9^{a}	27 ± 1.2^{a}	27 ± 0.9^{a}	27 ± 1.8^{a}	27 ± 1.5^{a}	27 ± 1.7^{a}	27 ± 0.9^{a}
Texture	Sandy-clay	Sandy-clay	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam	Sandy-clay loam	Sandy-clay
pH (H2O)	8.47 ± 0.1^{b}	8.41 ± 0.2^{b}	8.39 ± 0.2^{b}	8.07 ± 0.1^{a}	8.08 ± 0.4^{a}	8.04 ± 0.1^{a}	8.02 ± 0.1^{a}
pH (KCl)	6.99 ± 0.1^{a}	7.03 ± 0.2^{a}	6.99 ± 0.1^{a}	7.16 ± 0.2^{a}	7.17 ± 0.1^{a}	6.24 ± 0.2^{b}	6.52 ± 0.3^{a}
EC	301 ± 10^{a}	278 ± 13^{a}	299 ± 14^{a}	230 ± 10^{b}	233 ± 15^{b}	229 ± 13^{b}	222 ± 10^{b}
TP	332 ± 12^{c}	337 ± 10^{c}	365 ± 14^{b}	355 ± 12^{b}	398 ± 13^{a}	392 ± 11^{a}	352 ± 12a^{b}
MBC	1132 ± 22^{c}	1100 ± 18^{c}	1190 ± 16^{b}	1198 ± 12^{b}	1212 ± 12^{b}	1233 ± 12^{b}	1298 ± 12^{a}
CEC	11 ± 2^{a}	11 ± 1.5^{a}	14 ± 1.5^{a}	15 ± 0.9^{a}	16 ± 1.5^{a}	15 ± 1.5^{a}	14 ± 1^{a}
OC	1.4 ± 0.2^{a}	1.3 ± 0.1^{a}	1.5 ± 0.2^{a}	1.4 ± 0.2^{a}	1.4 ± 0.1^{a}	1.4 ± 0.1^{a}	1.4 ± 0.2^{a}
TN	0.16 ± 0.02^{a}	0.13 ± 0.02^{a}	0.12 ± 0.02^{a}	0.08 ± 0.02^{a}	0.12 ± 0.02^{a}	0.08 ± 0.02^{a}	0.08 ± 0.02^{a}
C/N	8.8± 1.1^{b}	1.3± 1.5^{c}	12.6± 1.8^{a}	16.6± 1.1^{a}	11.5± 1.5^{b}	16.3± 1.1^{a}	16.5± 1.7^{a}
OM	2.4 ± 0.3^{a}	2.2 ± 0.2^{a}	2.6 ± 0.3^{a}	2.4 ± 0.3^{a}	2.4 ± 0.2^{a}	2.4 ± 0.3^{a}	2.4 ± 0.2^{a}

DHA serving as an enzyme marker for oxidative activity in the soil, exhibited higher levels in soils treated with SB in both locations (Figure 3a and b). FDA displayed a similar trend to DHA in Motta, although it increased only at the lowest SB concentrations (Figure 3c and d). In contrast, no significant changes were observed for FDA in Lazzaro soil among the treatments. FDA reflects the activity of various hydrolytic enzymes, including esterases, proteases, and lipases (Nikaeen et al. 2015), and can serve as an indicator of soil biological activity, as noted by Komilis et al. (2011). Catalase, an antioxidant enzyme that increases in soil under stress conditions, protecting against oxidative damage by converting hydrogen peroxide into water and oxygen, decreased in SB-treated soils compared to the other treatments in both locations (Figure 3e and f). This suggests that the new fertilizer, at all concentrations, did not impose stress on the soil environment. β-glucosidase, a key enzyme in the decomposition of litter components associated with the carbon cycle, showed greater activity in the NPK treatment in Motta soil and in the SB treatments at 1.4 and 2.8 in Lazzaro soil (Table 4). Protease, responsible for the hydrolytic degradation of proteins, a crucial step in the nitrogen cycle, followed a similar trend to β- glucosidase in Motta soil, while in Lazzaro soil, it peaked in the presence of SB 4.2 (Table 4). Urease activity was highest in Motta soil when fertilized with NPK and in Lazzaro soil with HM and SB 2.8 (Table 4). The trends observed in fungi, bacteria, and actinomycetes mirrored those of the enzymes (Figures 4 and 5). Urease catalyzes the hydrolysis of urea into carbon dioxide and ammonia. Reduced urease activity can positively affect soil by preventing excessive urea hydrolysis, which could lead to ammonia loss through volatilization or rapid nitrification, shift in the microbial population, where the presence of sulfur has been reported to increase the percentage of sulphate-producing bacteria within the total bacterial population, at the expense of bacteria involved in nitrate and ammonium production, as indicated by Bouranis et al. (2019). Principal Component Analysis (PCA) revealed that ammonium and potassium, as expected, correlated with NPK and HM in both soils (Figure 6a and b).

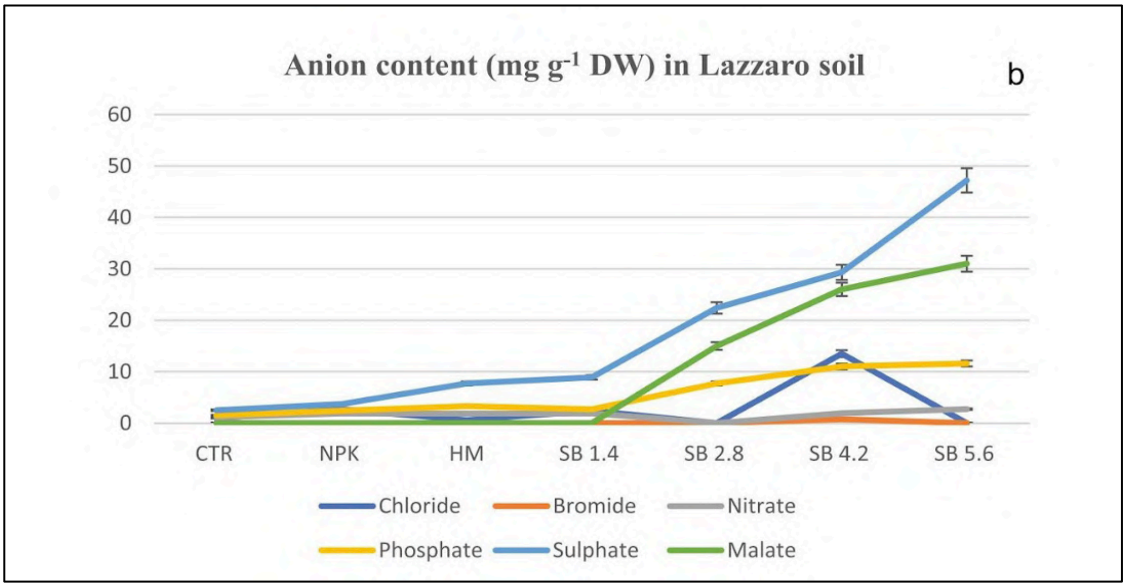

Figure 2. Anion content in Lazzaro (b) soils 6 months after the amendment with the different fertilizers. NPK= nitrogen:phosphorous:potassium; HM= horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6) and CTR= control, soil without fertilizer. Data are the means of six independent experiments and bars represent the standard error of the parameters analysed. Data are the means of three replicates (n = 18) ± standard errors

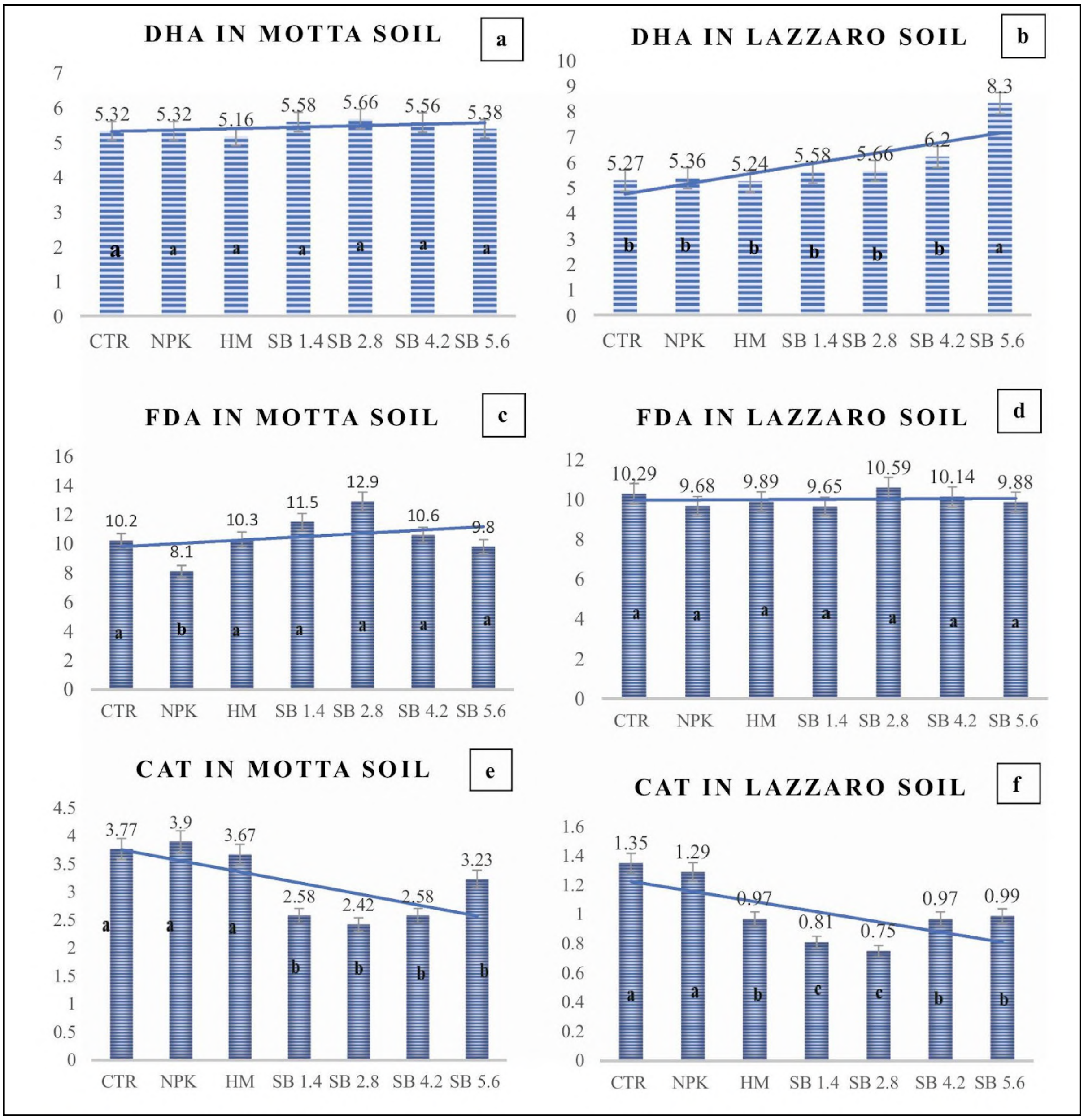

Figure 3 (a, b, c, d, e, f). dehydrogenase, (DHA), fluorescein diacetate hydrolase (FDA) Catalase (CAT) activities in Motta San Giovanni (a, c, e) and Lazzaro soils (b, d, f) 6 months after the amendment with the different fertilizers: CTR= control, soil without fertilizer; NPK= nitrogen:phosphorous:potassium; HM= horse manure; SB = sulphur bentonite + orange residue(at different concentrations: 1.4; 2.8; 4.2 and 5.6). Data are the means of six independent experiments and bars represent the standard error of the parameters analysed.

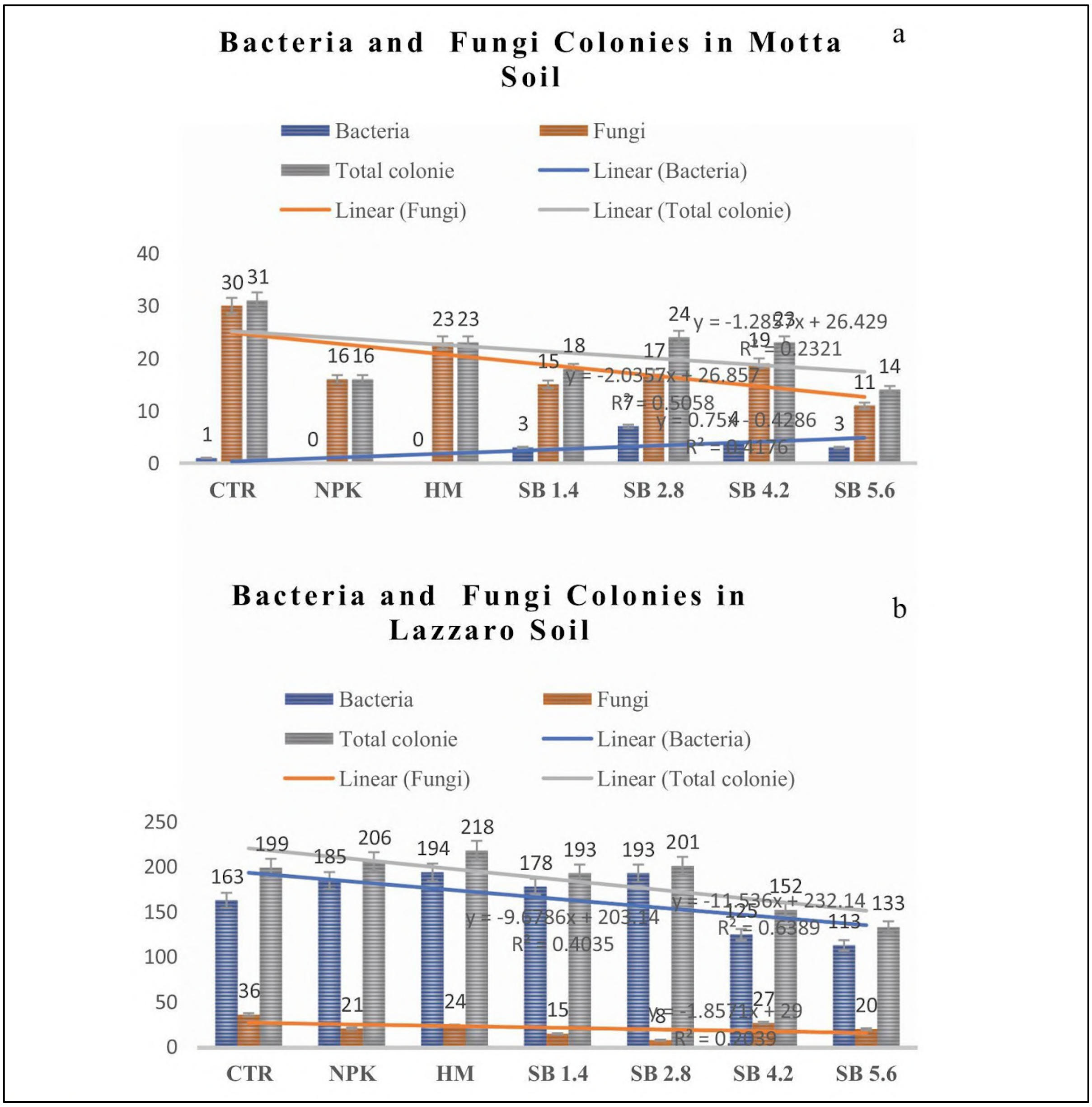

Figure 4. (a, b) bacteria (UFC 10^{-3}) and fungi (UFC 10^{-2}) colonies in Motta San Giovanni and Lazzaro soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM= horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6). Data are the means of six independent experiments and bars represent the standard error of the parameters analyzed.

Table 4. ß-glucosidase, protease and urease activities detected in Motta and Lazzaro soils 6 months after treatments with the different fertilizers. CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulfur bentonite + orange residue (at different concentrations 1.4; 2.8; 4.2 and 5.6)

	MOTTA			LAZZARO		
	ß-glucosidase	**Protease**	**Urease**	**ß-glucosidase**	**Protease**	**Urease**
CTR	514 ± 9^{e}	148 ± 9^{b}	350 ± 6^{d}	208 ± 6^{c}	166 ± 8^{b}	253 ± 9^{b}
NPK	709 ± 9^{a}	168 ± 5^{a}	403 ± 9^{a}	249 ± 10^{b}	158 ± 9^{b}	251 ± 5^{b}
HM	669 ± 11^{b}	168 ± 5^{a}	381 ± 7^{b}	209 ± 5^{c}	162 ± 8^{b}	273 ± 6^{a}
SB 1.4	559 ± 9^{d}	144 ± 5^{b}	319 ± 9^{e}	286 ± 7^{a}	165 ± 5^{b}	249 ± 9^{b}
SB 2.8	616 ± 11^{c}	176 ± 5^{a}	363 ± 8^{c}	291 ± 9^{a}	176 ± 11^{b}	265 ± 8^{a}
SB 4.2	648 ± 12^{b}	154 ± 7^{b}	267 ± 6^{f}	237 ± 7^{b}	210 ± 9^{a}	230 ± 7^{c}
SB 5.6	516 ± 11^{e}	154 ± 6^{b}	261 ± 9^{f}	244 ± 9^{b}	162 ± 5^{b}	197 ± 8^{d}

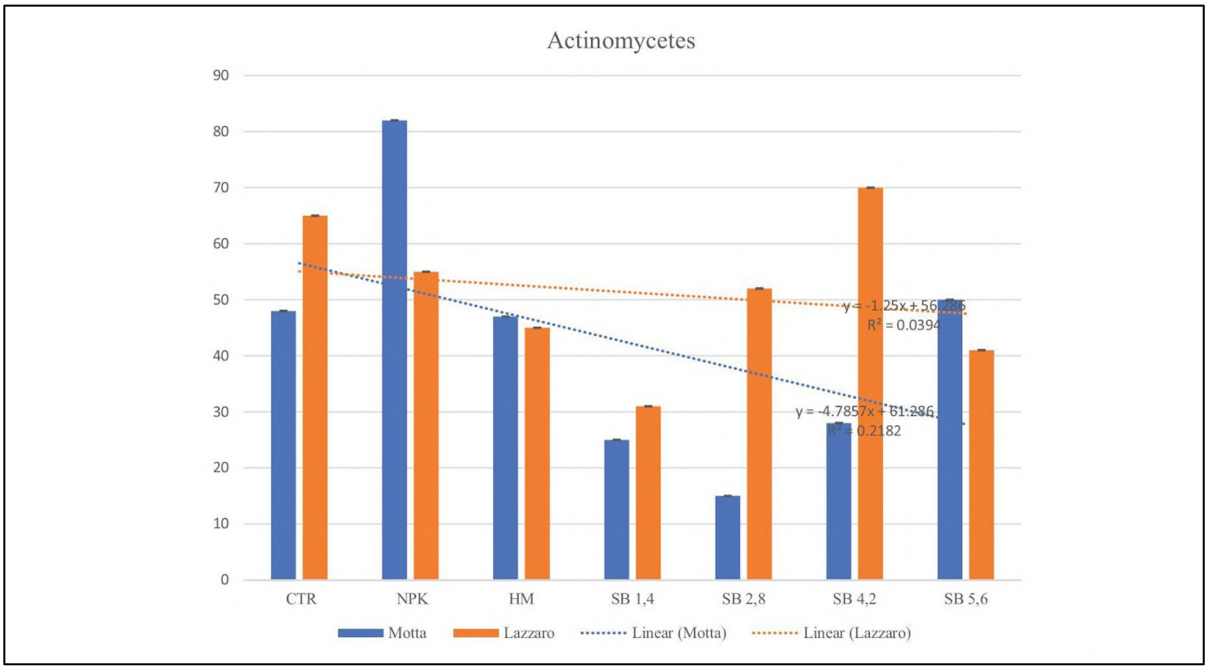

Figure 5. Actinomycetes detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6

In contrast, calcium was correlated only with SB. Magnesium in Motta soil showed a significant correlation with SB 4.2fertilization (Figure 6a), while in Lazzaro soil, it was linked to HM (Figure 6b). Anions exhibited correlations with SB fertilizations in both soils, with a few exceptions such as SB 1.4 in Motta and SB 1.4 and 2.8 in Lazzaro (Figure 7a, b). The PCA analysis concerning enzymes supported these observations, highlighting that protease, urease, and beta-glucosidase in Motta soil correlated with HM and NPK, while FDA and DHA were associated with SB 2.8 and 4.2. Catalase (CAT) exhibited a correlation only with the control (CTR) (Figure 8a). In Lazzaro soil, CAT correlated with NPK, HM, and CTR, while protease and beta-glucosidase correlated with SB 1.4, 2.8, and 4.2. FDA did not exhibit a correlation with any treatment, and DHA correlated only with SB 5.6 (Figure 8b). These findings highlighted significant variations in soil enzyme activities, primarily influenced by soil characteristics such as pH, cation exchange capacity (CEC), organic matter content, and microbial biomass, rather than fertilizer type. Differences in correlations with fertilizations were also observed for fungi, bacteria, and actinomycetes (Figure 9a and b). Bacteria in Motta soils were influenced by SB 2.8 and 4.2 (Figure 9a). Fungi correlated with HM, while actinomycetes were linked to NPK (Figure 9 A). In Lazzaro soil, bacteria were influenced by SB 2.8, NPK, and HM, while fungi and actinomycetes remained unaffected by the fertilization treatments (Figure 9b). These PCA results aligned with those for enzymes and confirmed that FDA and DHA, as indicators of soil biological activity, exhibited correlations with bacteria following a similar trend. In summary, the findings revealed that SB had a positive impact on both soils, albeit to varying degrees, influencing different soil properties. The concentrations at which maximum effectiveness was observed were 2.8 for Motta and 4.2 for Lazzaro, underscoring once again the pivotal role of soil characteristics in shaping the decomposition pathway of external substances introduced into the soil.

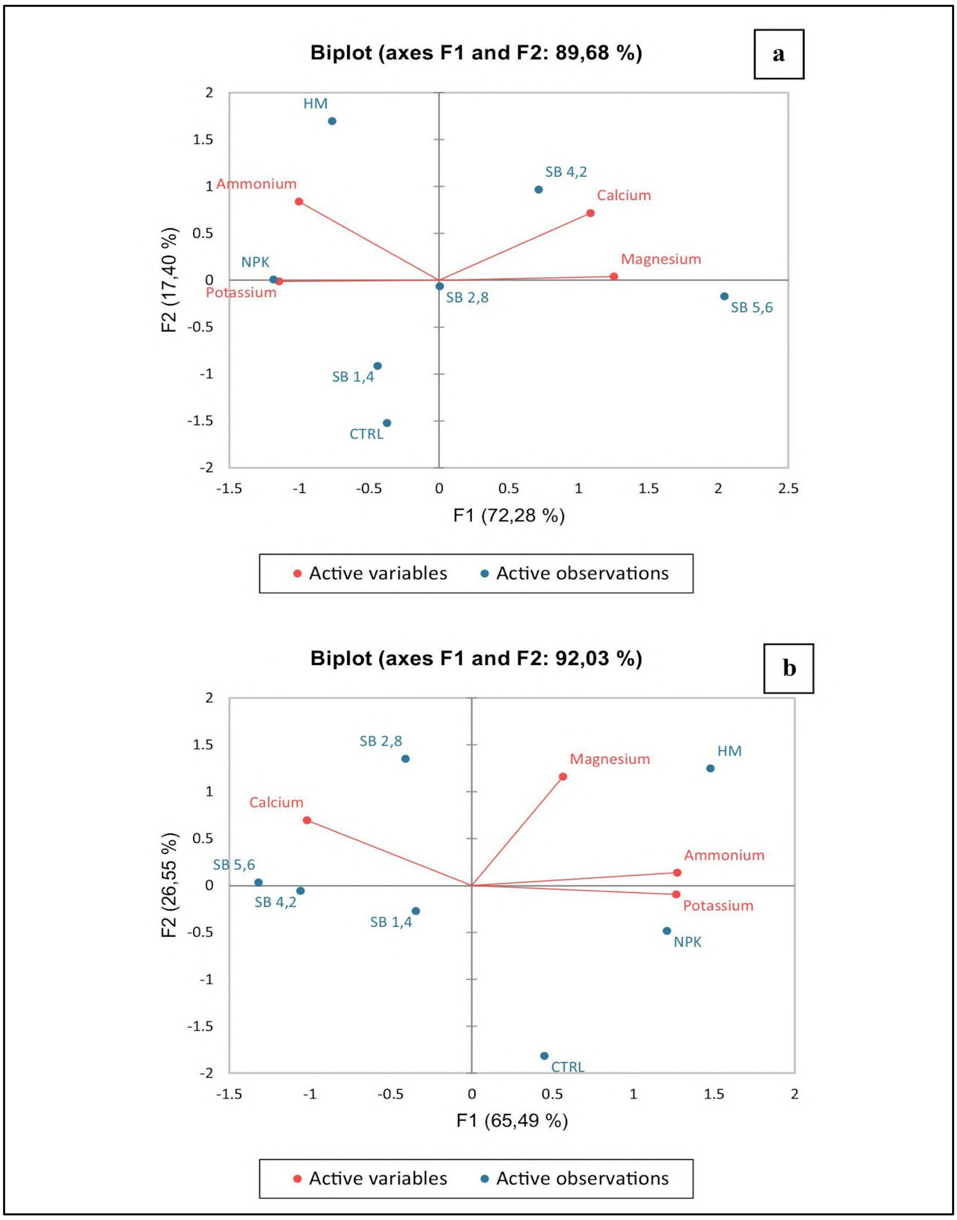

Figure 6 (a, b). PCA (principal component analysis) diagram of cations detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6)

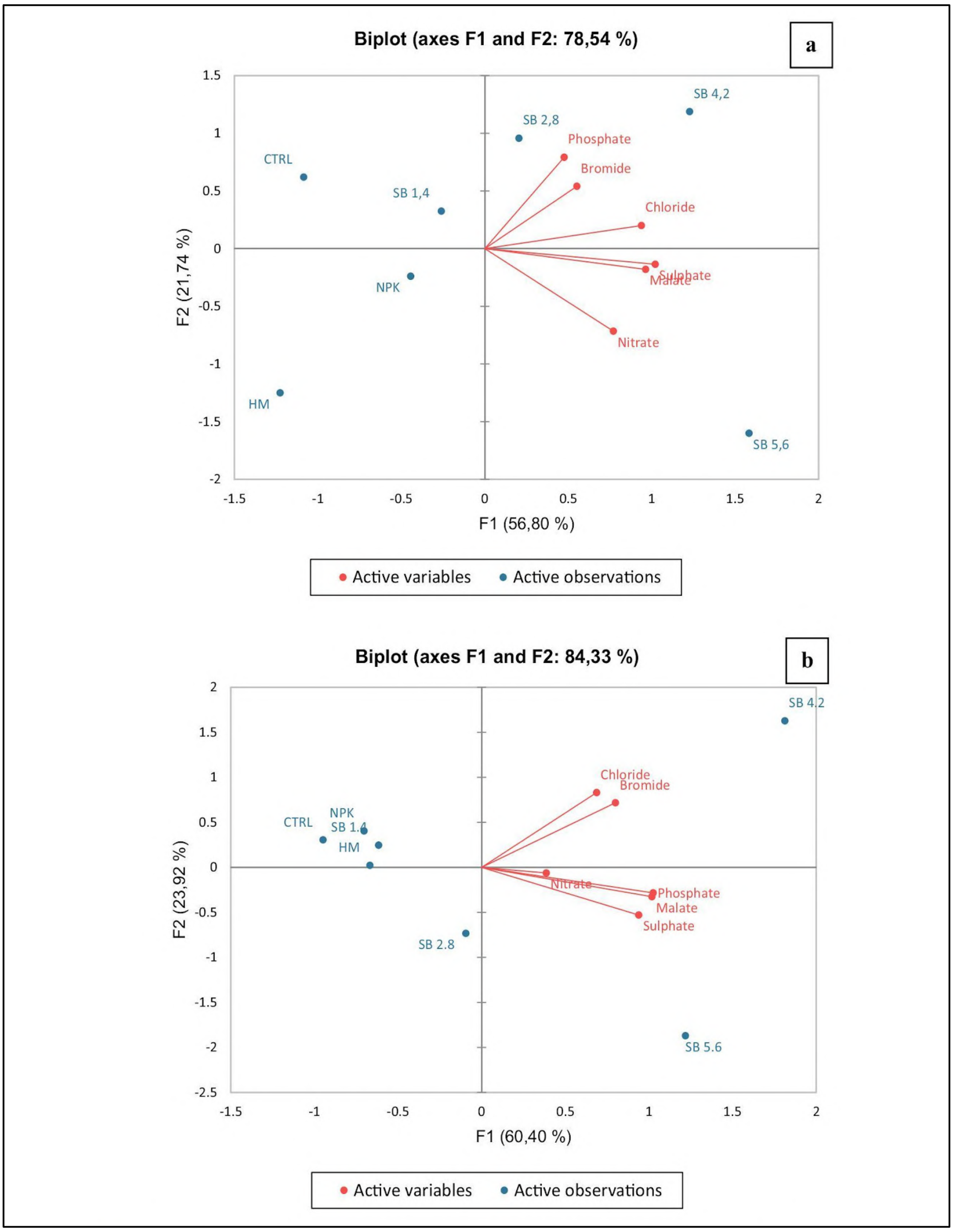

Figure 7 (a, b). PCA (principal component analysis) diagram of anions detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6

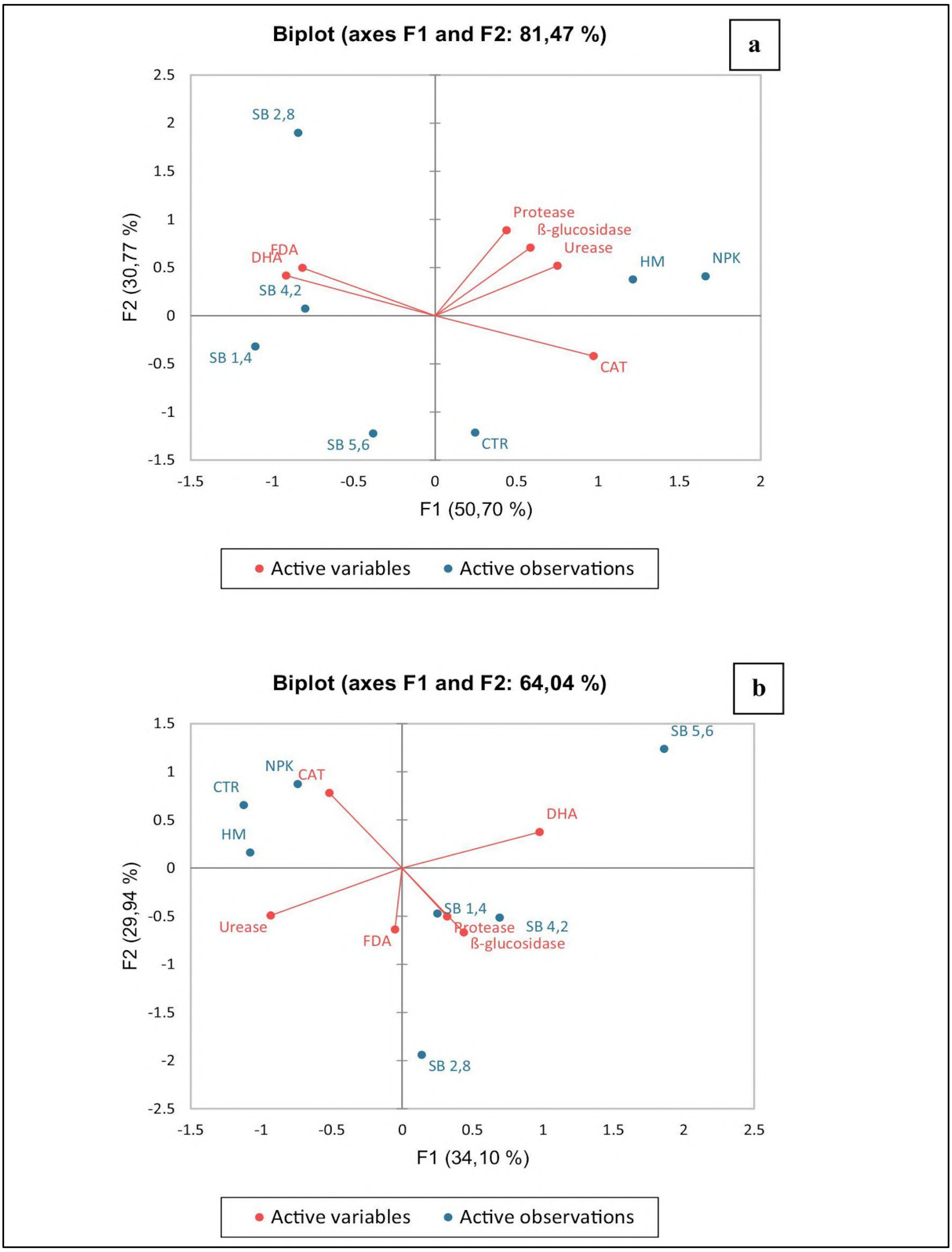

Figure 8 (a, b). PCA (principal component analysis) diagram of enzymes detected in Motta (a) and Lazzaro (b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK= nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).

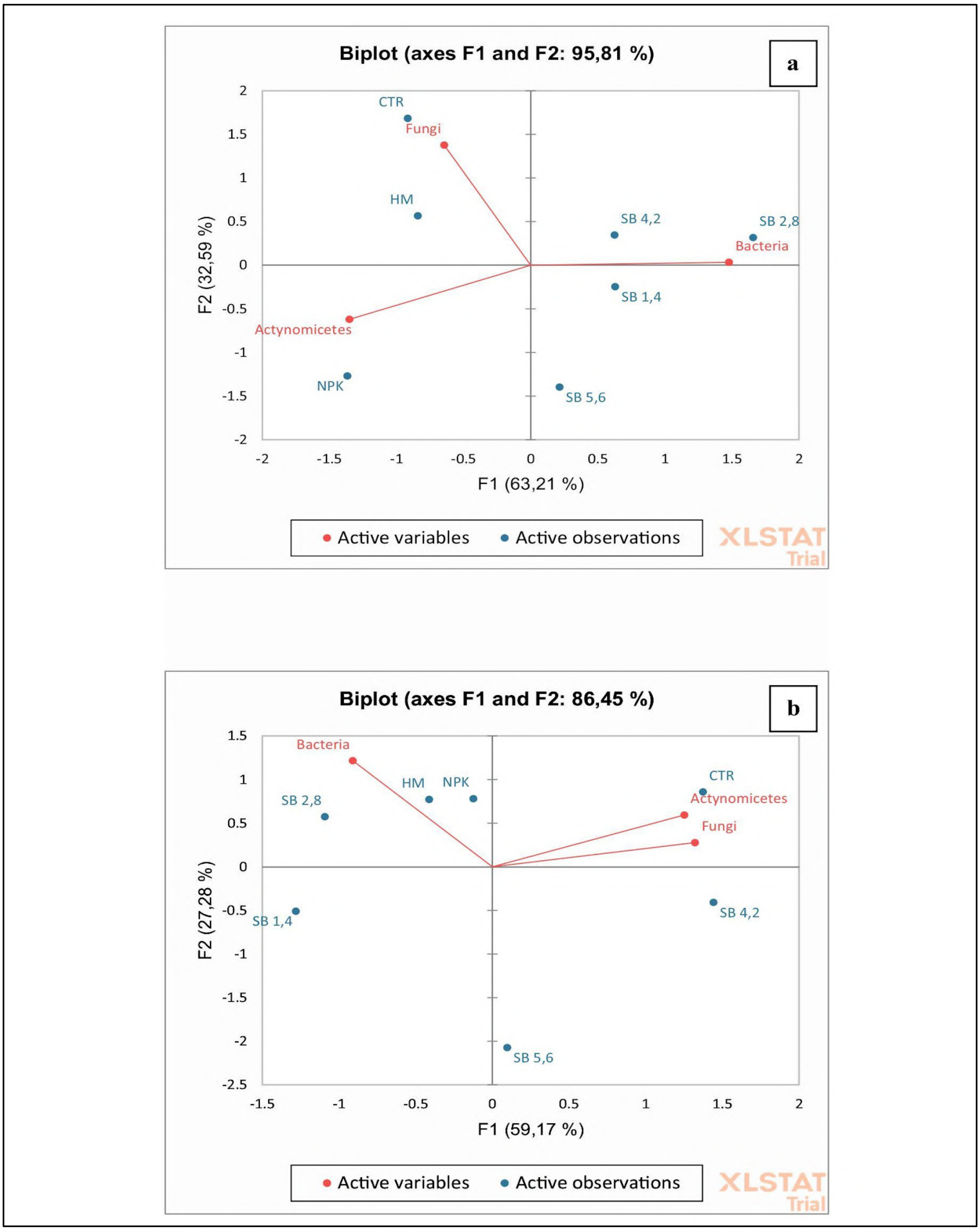

Figure 9 (a, b). PCA (principal component analysis) diagram of bacteria, fungi and actinomycetes found in Motta (a) and Lazzaro

(b) soils 6 months after the amendment with the different fertilizers: CTR = control, soil without fertilizer; NPK = nitrogen:phosphorous:potassium; HM = horse manure; SB = sulphur bentonite + orange residue (at different concentrations: 1.4; 2.8; 4.2 and 5.6).

4. Conclusions

This study stands as an innovative contribution, shedding light on the pivotal role that soil properties play in shaping the trajectory of fertilization and the long-term efficacy of fertilizers. Within the realm of soil organic matter (SOM), labile organic carbon emerges as a critical component, encompassing microbial biomass carbon (MBC) and enzymes. Among the various soil properties, these components were found to exert the most pronounced influence on fertilizer effectiveness. However, it's worth noting that the impact of the new fertilizer on the soil ecosystem, although varying in magnitude, consistently yielded positive outcomes in both soil types. In essence, this study underscores the potential benefits of transforming industrial and agricultural waste into fertilizers, offering both economic and environmental advantages by reducing waste disposal costs and lessening the reliance on mineral fertilizers in line with circular economy policies and strategies. The results unequivocally demonstrate an enhancement in soil quality when sulphur-based tablets are employed, surpassing the efficacy of commonly used organic and inorganic fertilizers. This holds particular significance in contemporary agriculture, especially within the organic farm paradigm, where continued dependence on traditional fertilizers is discouraged. In contrast to prior studies, this research accentuates the importance of characterizing soil properties to optimize the efficiency of fertilizer utilization.

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Chapter 4 - Comparative Study of Fertilizers in Tomato-Grown Soils: Soil Quality, Sustainability, and Carbon/Water Footprints.

Angela Maffia[1], Federica Marra[1], Francesco Canino[1] , Mariateresa Oliva[1], Carmelo Mallamaci[1], Giuseppe Celano[2], Adele Muscolo[1*].

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

[2] Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy

Correspondence: amuscolo@unirc.it

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Article

Comparative Study of Fertilizers in Tomato-Grown Soils: Soil Quality, Sustainability, and Carbon/Water Footprints

Angela Maffia [1], Federica Marra [1], Francesco Canino [1], Mariateresa Oliva [1], Carmelo Mallamaci [1], Giuseppe Celano [2] and Adele Muscolo [1,*]

[1] 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.); mariateresa.oliva@unirc.it (M.O.); carmelo.mallamaci@unirc.it (C.M.)
[2] Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy; gcelano@unisa.it
* Correspondence: amuscolo@unirc.it

Abstract: This manuscript delves into the pivotal role of sustainable agriculture in addressing environmental challenges and meeting the nutritional demands of a burgeoning global population. The primary objective is to assess the impact of a recently developed eco-friendly fertilizer, denoted as SBO, which arises from the blend of organic and mineral components derived from agricultural waste, sulfur, and residual orange materials. These elements are bound together with bentonite. This study compares SBO with distinct fertilizer treatments, including horse manure (HM) and nitrogen–phosphorous–potassium (NPK), on two diverse tomato-growing soils, each characterized by unique chemical and biological properties. Furthermore, the research extends to evaluate the environmental implications of these fertilizers, with a specific focus on their carbon and water footprints. Soils have been chemically and biochemically analyzed, and carbon and water footprints (CF and WF, respectively) have been assessed. The results reveal substantial enhancements in soil quality with the application of SBO fertilizer. Both soils undergo a transition towards near-neutral pH levels, an increase in organic matter content, and heightened microbial biomass. SBO-treated soils exhibit notably superior enzyme activities. The Life Cycle Assessment (LCA) results affirm the sustainability of the SBO-based system, boasting the lowest CF, while NPK demonstrates the highest environmental impact. Consistently, the WF analysis aligns with these findings, indicating that SBO necessitates the least water for tomato production. In summary, this study underscores the critical importance of adopting sustainable fertilization practices for enhancing soil quality and reducing environmental footprints in agriculture. The promising results offer potential benefits for both food production and environmental conservation.

Keywords: carbon footprint; soil fertility; soil quality; sustainability; water footprint

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1. Introduction

Food systems currently account for approximately 33% of total human greenhouse gas (GHG) emissions, representing a significant and increasing share of global emissions [1]. The emissions from agricultural activities and land use, estimated at around 12.0 Gt CO_2 per year, were quantified by the Food and Agriculture Organization (FAO) of the United Nations and the Intergovernmental Panel on Climate Change (IPCC) [1]. These emissions are expected to rise further due to the growing demand for food. One of the primary contributors to GHG emissions is the production of synthetic nitrogen (N) fertilizers used in crop cultivation. The application of these fertilizers is widely recognized as a major factor in the release of nitrous oxide (N_2O) emissions from agricultural soils. N_2O is a potent GHG, with a global warming potential 265 times greater than carbon dioxide (CO_2) and methane (CH_4) [2].

Moreover, in the early 1900s, a process was developed to mass-produce a compound containing ammonia, which greatly increased crop yields while utilizing less land. Ammonia is now one of the most extensively produced chemicals globally and is used in large quantities as a highly effective fertilizer [3]. However, excessive or improper application of nitrogen-based fertilizers can result in inefficient nitrogen uptake by plants, leading to an excess of nitrogen in the soil and subsequent N_2O emissions. According to the IPCC, the global use of synthetic nitrogen fertilizers has increased by 800% since the 1960s. The FAO estimates that this quantity is projected to increase by an additional 50% by the year 2050 [1].

The European Union's commitment to achieving a climate-neutral economy by 2050 is outlined in the European Green Deal, which emphasizes the role of every sector in this transition [4]. Soil, being a major carbon accumulator, stores twice as much carbon as the atmosphere and three times as much as terrestrial biomass [5]. Therefore, implementing appropriate and sustainable fertilization practices that enhance carbon storage and reduce CH_4 and N_2O emissions is crucial for agriculture to mitigate climate change while maintaining soil productivity [6].

Numerous researchers have demonstrated that long-term balanced fertilization, including sulfur (S) along with other macronutrients, can decrease N_2O emissions without compromising productivity, highlighting the significant role of elemental sulfur in improving and maintaining soil fertility [7–11].

Sulfur is recognized as a vital element for promoting optimal soil health and enhancing the availability of essential nutrients such as nitrogen, phosphorus, and potassium to plant roots. It deserves re-evaluation and consideration as a key component of soil fertility and productivity. Factors such as intensive cultivation practices, the use of sulfur-free fertilizers, and a decline in atmospheric sulfur depositions have contributed to sulfur deficiency in agricultural soils [7,8]. Various studies have emphasized that sulfur deficiency leads to reduced nitrogen utilization from fertilizers and the production of crop proteins with significantly lower levels of sulfur-containing amino acids, particularly methionine, which greatly influences the nutritional value of plants [9–11].

Kulczycki et al. [12] provided evidence of the positive effects of elemental sulfur on plant yields and soil properties in crops such as mustard, wheat, rapeseed, and corn. Further investigations revealed a negative association between the application of elemental sulfur and microbial activities in soils, leading to a significant decrease in microbial biomass carbon (MBC) and enzyme activities [13].

This decline in microbial biomass and enzyme functions could potentially be attributed to a scarcity of carbon substrates, particularly in soils with low organic matter content. Conversely, numerous studies demonstrated the stimulating effects of organic amendments on soil microbial biomass and activities responsible for nutrient release [14,15]. These findings have prompted researchers to explore the combination of sulfur and organic matter. In this regard, Tabak et al. [16] discovered that incorporating waste sulfur with organic materials facilitated the simultaneous enrichment of soil with readily available sulfur and organic matter. Moreover, Holatko et al. [17] conducted research showing that the utilization of elemental sulfur in conjunction with organic material, such as digestate, enhanced the availability of sulfur, resulting in increased yields and improved crop quality. Additionally, this combination was found to enhance the activity of soil microbes [18]. Our previous works [19–22] demonstrated that fertilizers produced by reusing different kinds of biomasses were effective for land restoration and crop improvement.

Tomato represents 13.5% of the world's vegetable production [23]. Several studies indicated that average GHG emissions for open-field tomato production were 0.2 and 2.0 kg CO^2 eq kg tomato^{-1} [24]. Hillier et al. [25] calculated the carbon footprint (CF) variation of different food crop production and found that applied N-fertilizer contributed most to variability. Results in Lee et al. [26] research found that the field-scale variability of GHG emissions is controlled primarily by biochemical parameters rather than physical parameters. The results demonstrated that variations in microbial activity, influenced by tillage and irrigation practices, lead to distinct levels and combinations of field-scale controls on GHG emissions.

Aldaya and Hoekstra [27] analyzed Italian industrial tomato production that had a water footprint (WF) equal to 114 m^3 t^{-1} (30% green, 50% blue, and 17% grey). Comparing these results with Chapagain and Orr [28], the blue component was in accordance with them, while green and grey were much higher in the Italian productive system due to different weather conditions and fertilizer inputs.

Simultaneous assessment of carbon and WF for agri-food products is desirable to provide better insights into key environmental issues than using either indicator in isolation [29]. These indicators measure the potential impact of a product throughout its entire life cycle in terms of GHG emissions and the consumption and degradation of water resources. The WF quantifies the volume of freshwater consumed or polluted (through evaporation or product incorporation) within a given time, while the CF indicates the Global Warming Potential over a defined period. The aim of this study was to evaluate the effects of agro-industrial waste-based fertilizer on the quality, CF, and WF of two soils used for growing industrial tomatoes (var. Big Rio F1), both in a climatic chamber and open field, compared to commonly used chemical and organic fertilizers.

2. Materials and Methods

2.1. Agro-Industrial Waste-Based Fertilizer Manufacturing

The fertilizer is composed of 85% elemental sulfur (S) pelletized with 10% bentonite clay (as the carrier and inert support) and 5% orange residue. After the pelletized phase, the mixtures were introduced in a rotary pastillator system, which deposits the liquid pads of the above-listed ingredients opportunely mixed on a heat exchanger in continuous steel tape for the solidification of the pods. In the end, pads with a diameter of 3/4 mm were obtained.

2.2. Soil Treatment

Fertilizers were tested on Tomato plants, variety Big Rio F1, grown in 30 cm diameter pots containing 9 kg of soil. For the trial, we conducted three replicates for each soil treatment. The experimentation took place in a climatic chamber to simulate the optimal environmental conditions for plant growth. The climatic conditions set in the climatic chamber for the germination phase were 25 °C, 70% humidity, and 800 Lux. For the vegetative development phase of the plants, climatic parameters were set following the climatic data from May until the end of August, considering that the vegetative cycle is 120 days for this variety. Two fertilizations were carried out: one at the beginning of the crop cycle and one at complete flowering. The fertilizer dosage to be used was chosen based on results obtained previously on various soils and different crops [19,20]. The fertilizer amount used for each treatment and pot is:

(A) 1.2 g of synthetic fertilizer NPK (15-15-15) in which there are 0.18 g of N, 0.18 g of P_2O_5 and 0.18 g of K_2O.
(B) 13 g of organic fertilizer, in which there are 0.26 g of N, 0.26 g of P_2O_5, and 0.195 g of K_2O. This fertilizer contains bovine, equine, sheep, and poultry manure mixed with litter, calcium sulfate, and olive pomace dust, which accounts for 50% of the total composition percentage.
(C) 1.4 g of fertilizer sulfur–bentonite + orange residue.

2.3. Data Collection and Analysis

Soil samples were taken at 20 cm depth for each pot at the beginning of the trial, before planting the tomato seedling, and then six months later, after harvesting and subsequent removal of plants.

Data are expressed as means of three analyses for each treatment. Analysis of variance was carried out for all the data sets. One-way ANOVA with Tukey's Honestly. Significant difference tests were carried out to analyze the effects of fertilizers on each of the various parameters measured; ANOVA and *t*-test were carried out using XLStat. Effects were significant at $p \leq 0.01$. To explore. Relationships among different fertilizers on soil parameter datasets we analyzed using Principal Component Analysis (PCA) with XLStat.

2.4. Soil Physical, Chemical, and Biochemical Analysis before and after Treatment

Potted soils before treatment were analyzed for physical and chemical properties. Soils were taken in the *Grecanica* area (Tables 1 and 2), specifically in the municipalities of Reggio di Calabria (RC) (soil 1) and Motta San Giovanni (RC) (soil 2). These soils were classified as sandy-loam soil (soil 1) and sandy-clay soil (soil 2) based on the FAO soil classification system [30]. Soil texture was assessed using the hydrometer method [31]. The pH was measured in distilled water (soil/solution ratio 1:2.5) with a glass electrode. Electric conductivity (EC) was determined in distilled water using a 1:5 soil/water suspension mechanically shaken at 15 rpm for 1 h and then detected with a Hanna instrument conductivity meter. Cation exchange capacity (CEC) was determined using the Mehlich methodology [32]. Organic carbon was assessed with the dichromate oxidation method [33] and transformed into organic matter by multiplying by 1.72; Total nitrogen (TN) was measured with the Kjeldahl method [34]. C/N was determined as a carbon/nitrogen ratio. Water-soluble phenols (WSPs) were extracted in triplicate, as reported by Kaminsky et al. [35]. Gallic acid was used as a standard, and the concentration of WSP compounds was expressed as Gallic Acid Equivalents (μg GAE g^{-1} d.s). MBC was determined using the chloroform fumigation-extraction procedure on fresh soil. [36] Fumigated and unfumigated soil sample extracts were used to detect soluble organic C using the methods of Walkley and Black [33].

Table 1. Physical and chemical properties of soils before fertilization. Data are the means of three replicates ± standard deviation.

	SOIL 1	SOIL 2
Skeleton (%)	45 ± 0.01	21 ± 0.02
Sandy %	65 ± 0.02	50 ± 0.02
Clay %	23 ± 0.12	27 ± 0.13
Silt %	12 ± 0.23	23 ± 0.24
Textural Class	Sandy-loam	Sandy-Clay-loam
Moisture %	18 ± 0.4	32 ± 0.3
S.S (%)	82 ± 0.4	68 ± 0.3
pH (H_2O)	8.5 ± 0.32	8.3 ± 0.43
pH (KCl)	7.8 ± 0.53	7.3 ± 0.34
EC (μS/cm)	107.3 ± 12.3	302 ± 11.5
CEC (cmol$^{(+)}$ kg^{-1})	21.57 ± 13.5	27.71 ± 17.8
TOC %	1.78 ± 0.13	1.98 ± 0.42
TN %	0.19 ± 0.14	0.2 ± 0.15
C/N	9.37 ± 0.13	9.9 ± 0.17
SOM %	3.07 ± 0.13	3.41 ± 0.13
WSP (μg GAE g^{-1} d.s)	56.1 ± 14.5	85.62 ± 18.5
MBC (μg C g^{-1} soil)	896 ± 10.6	941 ± 1.4

Table 2. Soil enzymatic activities before fertilization. Dehydrogenase (DHA, μg INTF g^{-1} d.s h^{-1}). Catalase activity (CAT O_2/3 min/g d.s). Fluorescein diacetate hydrolase (FDA, μg fluorescein g^{-1} d.s). Urease (URE, N-NH_4/g d.s/3 h). beta-glucosidase (ß-GLU, μg para-nitrophenol (p-NP) g/h). Protease (PRO μg Tyrosine g d.s 2 h). Data are the means of three replicates ± standard deviation.

	SOIL 1	SOIL 2
DHA	1.31 ± 0.67	2.83 ± 0.53
CAT	1.54 ± 1.45	3.85 ± 1.76
FDA	8.93 ± 0.36	15.10 ± 1.03
ß-GLU	514 ± 0.24	348 ± 0.54
PRO	167 ± 1.65	157 ± 2.06
URE	312 ± 0.12	289 ± 0.65

Dehydrogenase (DHA) activity was detected with iodonitrotetrazolium chloride according to the von Mersi and Shinner method [37]. Catalase activity (CAT) was detected using the method of Kuush et al. [38], measuring the absorbance during the conversion of H_2O_2 to oxygen and water. The decrease in the absorbance was measured at 240 nm, utilizing the extinction coefficient of 39.4 M^{-1} cm^{-1}. Fluorescein diacetate hydrolase (FDA) activity was determined according to the method of Adam and Duncan [39]. Beta-glucosidase activity was tested as reported by Valášková et al. [40] with the modifications reported in Muscolo et al. [19]. Soil (1 g fresh weight) was placed into a plastic tube and treated with 4 mL of modified universal buffer (MUB, pH 6). The reaction mixture contains 0.16 mL of 1.2 mM PNP substrate (p-nitrophenyl-β-D-glucoside) in 50 mM sodium acetate buffer (pH 5.0) and 0.04 mL of the sample. Reaction mixtures were incubated at 40 °C for 20–120 min. After incubation, the reaction was stopped, and the yellow color from the p-nitrophenol was developed by the addition of 0.1 mL of 0.5 M sodium carbonate; the p-nitrophenol absorbance was measured on a spectrophotometer at a wavelength of 400 nm and quantified by comparison with a standard curve. Protease activity was detected, as reported by Sidari et al. [41]. Two ml of phosphate buffer (0.1 M, pH 7.1) and 0.5 mL of 0.03 M N-a-benzoyl-L arginine amide (BAA) have been added to 1 g of wet soil. The mixture was incubated at 37 °C for 1 h and 30 min, then diluted to 10 mL with distilled water. The ammonium concentration was detected with an ammonium selective electrode (CRISON, micro-pH 2002). Urease activity was determined following the method of Kandeler and Gerber [42], with a few modifications, as reported in Sidari et al. [41]. Five grams of fresh soil were mixed with 2.5 mL of urea (80 mM) and 20 mL 0.1 M borate buffer at pH 10.0. After 2 h in an orbital shaker at 37 °C, 2.5 mL of urea were added to the control. 30 mL of KCl (2 M) were instead added to both the sample and, and shake for 30 min. One ml of the filtered solutions was mixed with 9 mL of distilled water, 5 mL of sodium/salicylate solution, and 2 mL of dichloroisocyanuric acid. Ammonium concentrations were determined at 690 nm by using a calibration curve. The results are reported as μg N-NH_4/g d.s/3 h. [41].

2.5. Environmental Impact: Carbon and Water Footprint

The study was carried out in Grecanica Area, specifically in Motta San Giovanni, province of Reggio Calabria. The main features of the studied system were collected through visits to farms and direct interviews with farmers using a specific collection sheet. The farm carried out the treatments by subdividing four experimental plots, carrying out the treatments described in Section 2. The farm grew tomatoes of the Big Rio F1 variety, with a planting density of 3 plants/m^2, with a sprinkler irrigation system. Insecticide treatment (with PRIMOR 500) and fungicide treatments (with DARAMUN) were the same for all experimental plots, as were soil tillage and harvesting. The only difference between the experimental plots was the fertilization. The farm inputs and outputs used in the analyzed systems are reported in Table 3.

Table 3. Farm inputs and outputs used in the analyzed systems.

	CTR	A	B	C
Fertilizers (kg ha^{-1})				
NPK		170		
Horse Manure			430	
Sulfur Bentonite + Orange waste				476
Chemicals (kg ha^{-1})				
Primor 50	0.75	0.75	0.75	0.75
Daramun	4	4	4	4
Human labour (h ha^{-1})	45	46	48	46
Machinery (h ha^{-1})	5	10	12	10
Diesel (kg ha^{-1})	10	13	15	13
Water (m^3 ha^{-1})	750	750	750	750
Electricity (kWh kg^{-1})	0.13	0.14	0.15	0.15
Production (t ha^{-1})	47	57.5	58	58.4

CTR = Control unfertilized soil; A = nitrogen–phosphorous–potassium; B = horse manure; C = sulfur bentonite + orange residue.

2.5.1. Carbon Footprint

The LCA approach, according to the ISO 14040:2006 [43], was used to estimate environmental impacts with a focus on Global Warming (GWP 100a) for the CF. This methodology contains four stages: goal and scope definition, life cycle inventory, life cycle impact assessment, and interpretation [44]. The system boundaries, as shown in Figure 1, start with the planting of tomato plants and end with harvesting. Following the PCR 2010:07, "Arable and vegetative crops" corresponds to CORE processes, which are the farming phases: transport of plants, acceptance of plants, planting, and harvest. In accordance with the PCR, [45] this study does not include manufacturing of buildings and capital goods other than agricultural machinery, business travel of personnel, travel to and from work by personnel, and research and development activities.

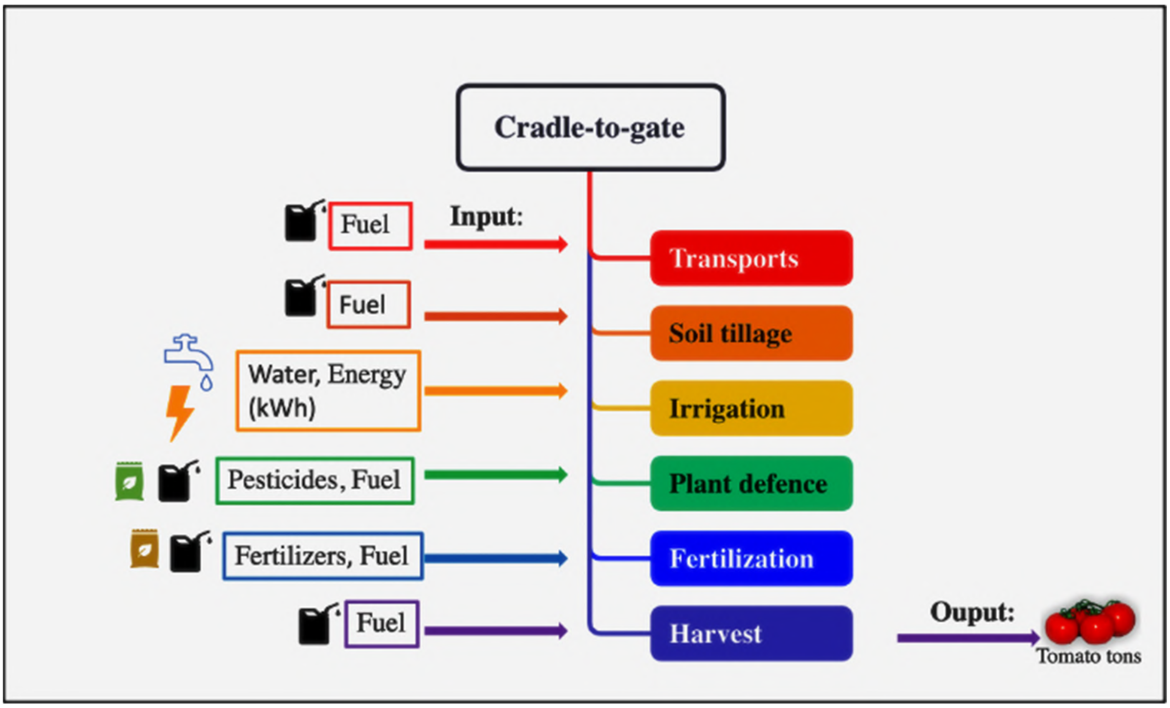


Figure 1. System boundaries of CORE process of tomato production.

Furthermore, due to the lack of data, the analysis did not consider input transport (fertilizers and pesticides) from the place of production to the field. All the inputs used in the agricultural phase (fertilizers, pesticides, fuels, and various materials utilized during the harvesting) were considered. Primary data were collected in situ during the 2021–2022 agricultural year, from May to October, using a data collection sheet on a farm that carried out the experimentation in collaboration in the open field located in Motta San Giovanni (RC). The sample used for data collection is, therefore, homogeneous, consisting of a single farmer who divided his field into several experimental plots fertilized with different types of fertilizer (synthetic, organic, and sulfur bentonite orange waste-based fertilizer), the quantities of which are shown in Table 3 where all inputs and outputs (tons of tomato) of the production process are described. Direct emissions from fuel and lubricant were taken from SimaPro's LCI database and calculated as reported by Pergola et al. [46].

Ammonia volatilization from mineral fertilizers application (namely NPK for system A) to soil incorporation, evaluated taking into account the type of mineral fertilizer and the geographical location of the olive system, was equal to 2% of the applied $N\text{-}NH_4$. So, N_2O emissions from fertilizers were computed considering the emission factor equal to 0.0125. Emission of synthetic pesticides to air, surface water, groundwater, and soil was estimated according to the methodology suggested by Hauschild [47]. These estimates considered both site conditions (soil organic matter, texture, climate, etc.) and physicochemical characteristics of the active ingredients (vapor pressure, half-life determined by photolysis, half-life in soil, adsorption coefficient to organic material in soil).

The impact assessment was performed using SimaPro 9.02, with the problem-oriented LCA method (CML-IA Baseline V3.06/EU25 + 3, 2000) developed by the Institute of Environmental Sciences of the University of Leiden [48]. The following impact categories were considered according to the selected method: abiotic depletion (AD); abiotic depletion (fossil fuels) (AD fossil fuels); global warming potential (GWP) or climate change; photochemical oxidation (PO); ozone layer depletion (ODP); human toxicity (HT); freshwater aquatic ecotoxicity (FWE); marine aquatic ecotoxicity (MAE); terrestrial ecotoxicity (TE); air acidification (AA) and eutrophication (EU).

2.5.2. Water Footprint

For this study, WF was performed as established by Hoekstra et al., 2011 [49] in "The Water Footprint Assessment Manual," which defines the guidelines for the WF. The peculiarity of this methodology is the division of the WF into three components: blue, green, and grey. Blue, green, and grey components were calculated through the CROPWAT model. The total WF is given by the sum of components according to Equation (1):

$$WF_{total}: WF_{green} + WF_{Blue} + WF_{grey} \quad (1)$$

For the WF green reported in Equation (3) and blue reported in Equation (4), the values for crop water requirement (CWR, $m^3\ ha^{-1}$) are calculated using the formulas below:

$$WF_{green} = CWU_{green}/\text{crop yield} \quad (2)$$

$$WF_{blue} = CWU_{blue}/\text{crop yield} \quad (3)$$

The green and blue components of CWU ($m^3\ ha^{-1}$) reported in Equations (4) and (5), respectively, were calculated by the accumulation of daily evapotranspiration (ET mm/day) over the whole growing season:

$$CWU_{green} = 10 \times \sum_{d=1}^{d=harvesting} ETgreen \quad (4)$$

$$CWU_{blue} = 10 \times \sum_{d=1}^{d=harvesting} ETblue \quad (5)$$

ETgreen represents green water evapotranspiration, and ETblue instead of blue water evapotranspiration [49]. The summation is performed over the period from the day of planting (day 1) to the day of harvest. The green and blue water evapotranspiration has been estimated using the CROPWAT model developed by the Food and Agriculture Organization of the United Nations [50], which is based on the method described by Allen et al. [51].

The CROPWAT model offers two different options to calculate evapotranspiration: the 'crop water requirement option' (assuming optimal conditions) and the 'irrigation schedule option' (including the possibility to specify actual irrigation supply in time [52] and for this study, we use the first option. According to Pellegrini et al. [53]:

- ETgreen was calculated as the minimum of Crop WaterRequirement (CWR, mm $year^{-1}$) and effective precipitation (Peff, mm $year^{-1}$).
- ETblue was estimated from Irrigation Requirement (IR) rates as the minimum between IR (m^3 $year^{-1}$) and the irrigation volume (Ieff, m^3 ha^{-1} $year^{-1}$). [53].
- IR was calculated as a constant value for the analyzed systems according to the following equation: IR = max (0; CWR-Peff).

In the CROPWAT 8.0 software, after entering the input data related to climate data of Reggio Calabria in the year 2021–2022, crop Kc, rainfall, and soil characteristics, we obtained the CWR needed to calculate the green and blue WF, as shown in Table 4.

Table 4. Crop water requirement obtained from CROPWAT 8.0.

Month	Decade	Stage	Kc	ETc	ETc	Eff Rain	Irr. Req.
			coeff	mm/day	mm/dec	mm/dec	mm/dec
May	2	Init	0.6	2.24	13.4	8.4	6.4
May	3	Init	0.6	2.45	26.9	14.1	12.9
Jun	1	Init	0.6	2.7	27	14.6	12.3
Jun	2	Deve	0.64	3.12	31.2	14.3	16.9
Jun	3	Deve	0.78	3.76	37.6	13.6	24
Jul	1	Deve	0.93	4.31	43.1	12.4	30.8
Jul	2	Deve	1.07	4.9	49.	11.4	37.8
Jul	3	Mid	1.18	5.9	64.9	12.8	52
Aug	1	Mid	1.18	6.61	66.1	14.9	51.2
Aug	2	Mid	1.18	7.14	71.4	16.3	55.2
Aug	3	Mid	1.18	6.65	73.1	15.6	57.6
Sep	1	Late	1.17	6.01	60.1	14.6	45.5
Sep	2	Late	1.07	5.1	51	14.1	36.9
Sep	3	Late	0.95	3.9	39	13.9	25
Oct	1	Late	0.85	2.83	17	7.3	10.9

Following Hoekstra et al. 2011 [49], the grey component of WF was calculated as:

$$\text{WF product, grey} = [(\alpha \times AR)/(C_{max} - C_{nat})]/Y \quad (6)$$

where:

- AR is the chemical application rate to the field per hectare (kg ha^{-1});
- α is the leaching-run-off fraction;
- C_{max} is the maximum acceptable concentration for the pollutant considered (kg m^{-3});
- C_{nat} is the natural concentration for the pollutant considered (kg m^{-3});
- Y is the crop yield (t ha^{-1}).

For the grey component of the WF, only nitrogen fertilizers have been considered, according to Hoekstra et al., 2011 [49], because Nitrogen represents an important source of pollution in Europe, as can be seen from the Nitrates Directive 1990 [54]. The chemical application rate (AR) (kg ha^{-1}) for the different cultivation systems used to calculate this footprint is reported in Table 3. From Legislative Decree 152/2006, the acceptable limit value for nitrogen was found to be 15 mg/L (C_{max}), the maximum acceptable concentration (C_{nat}), following Hoekstra et al. [49] was considered to be 0, and a leaching factor (α) 0.1 was considered for all cultivation systems.

By applying Equations (2)–(7), it was possible to obtain the blue, green, and grey WF values over the reference period 2022–2023, specifically from May 2022 to October 2022 (Table 4), referring to the same inventory analysis used for the CF (Table 3)

3. Results

3.1. Effect of Different Fertilizers on Soil Quality

The chemical properties of soil 1, assessed six months after the treatments, exhibited significant variations (Table 5). Notably, there were no significant changes in soil texture. However, in the soils treated with SBO, notable reductions were observed in both pH in water and KCl levels. Simultaneously, there was an increase in electrical conductivity (EC), suggesting the potential for an enhanced mineralization process with ion release. Additionally, the organic matter content, C/N ratio, CEC, and microbial biomass all experienced increases (Table 5).

Table 5. Physical and chemical properties of soil 1 and soil 2 six months after the treatments with the different fertilizers: CTR = Control unfertilized soil; A = nitrogen–phosphorous–potassium; B = horse manure; C = sulfur bentonite + orange residue.

	CTR	A	B	C	CTR	A	B	C
	Soil 1				**Soil 2**			
Skeleton (%)	45 [a*]	45 [a]	45 [a]	45 [a]	21 [a]	21 [a]	21 [a]	21 [a]
Sandy %	65 [a]	65 [a]	65 [a]	65 [a]	50 [a]	50 [a]	50 [a]	50 [a]
Clay %	23 [a]	23 [a]	23 [a]	23 [a]	27 [a]	27 [a]	27 [a]	27 [a]
Loam %	12 [a]	12 [a]	12 [a]	12 [a]	23 [a]	23 [a]	23 [a]	23 [a]
Textural Class	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-loam	Sandy-Clay-loam	Sandy-Clay-loam	Sandy-Clay-loam	Sandy-Clay-loam
pH (H_2O)	8.31 [a]	8.76 [a]	8.01 [b]	7.82 [b]	8.22 [a]	8.43 [a]	7.89 [ab]	7.21 [b]
pH (KCl)	7.70 [a]	7.85 [a]	7.75 [a]	7.58 [b]	7.21 [a]	7.11 [ab]	7.42 [a]	6.98 [b]
EC (µS/cm)	106 [b]	123 [ab]	132 [a]	142 [a]	298 [a]	291 [ab]	267 [b]	326 [a]
CEC	20.65 [b]	22.87 [b]	23.65 [ab]	28.56 [a]	26.61 [b]	24.23 [b]	26.45 [b]	31.56 [a]
TOC %	1.67 [b]	1.45 [b]	2.02 [a]	2.03 [a]	1.51 [b]	1.25 [b]	1.98 [a]	2.12 [a]
TN %	0.14 [a]	0.17 [a]	0.16 [a]	0.12 [ab]	0.12 [b]	0.21 [a]	0.19 [a]	0.15 [b]
C/N	11.93 [b]	8.53 [c]	12.63 [b]	16.92 [a]	12.58 [a]	5.95 [c]	10.42 [b]	14.13 [a]
SOM %	2.88 [b]	2.50 [b]	3.48 [a]	3.50 [a]	2.60 [ab]	2.16 [b]	3.41 [a]	3.65 [a]
WSP (µg GAE * g^{-1} d.s)	55.68 [a]	55.23 [a]	52.45 [b]	51.53 [b]	82.67 [b]	79.81 [b]	91.76 [a]	97.23 [a]
MBC (µg C g^{-1} f.s)	901 [b]	876 [b]	926 [a]	976 [a]	945 [b]	965 [b]	1023 [ab]	1198 [a]

* Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). Values are the mean of three replicates (n = 12).

Conversely, in the soils treated with SBO, the phenol content and catalase activity decreased compared to all other treatments, while other enzyme activities increased. A strong positive and significant correlation was observed between MBC, organic matter, pH in KCl, C/N ratio, WSPs, FDA hydrolase, dehydrogenase protease, and urease activities. MBC correlated inversely with catalase activity and did not correlate with beta-glucosidase (Table 6).

Table 6. Enzymatic activities of Soil 1 and Soil 2 six months after treatments with the different fertilizers. CTR = Control. soil without fertilizer; A = nitrogen:phosphorous:potassium; B = horse manure; C = sulfur bentonite + orange residue. Dehydrogenase (DHA, µg INTF g^{-1} d.s h^{-1}), Catalase activity (CAT. O_2/3 min/g d.s), Fluorescein diacetate hydrolase (FDA, µg fluorescein g $^{-1}$ d.s), beta-glucosidase (ßGLU, µg para-nitrophenol (p-NP) g/h), Protease (PRO µg Tyrosine/g d.s/2 h), Urease (URE, N-NH_4/g d.s/3 h).

	CTR	A	B	C	CTR	A	B	C
		Soil 1				Soil 2		
DHA	1.29 ^{b*}	1.34 ^{b}	1.59 ^{ab}	2.01 ^{a}	2.69 ^{b}	3.1 ^{ab}	3.5 ^{a}	3.8 ^{a}
FDA	8.56 ^{b}	8.51 ^{b}	9.01 ^{a}	9.34 ^{a}	14.50 ^{b}	15.51 ^{b}	17.01 ^{a}	19.14 ^{a}
CAT	1.34 ^{a}	1.51 ^{a}	1.01 ^{b}	0.96 ^{b}	2.84 ^{a}	2.51 ^{a}	2.41 ^{a}	1.96 ^{b}
ßGLU	510 ^{a}	546 ^{a}	555 ^{a}	557 ^{a}	355 ^{b}	365 ^{b}	372 ^{ab}	401 ^{a}
PRO	165 ^{b}	157 ^{b}	167 ^{b}	212 ^{a}	145 ^{b}	157 ^{b}	177 ^{a}	201 ^{a}
URE	298 ^{b}	312 ^{ab}	351 ^{b}	365 ^{b}	258 ^{b}	297 ^{a}	281 ^{a}	279 ^{a}

* Different letters in the same row indicate significant differences (Turkey's test $p \leq 0.05$). The data are the mean of three replicates (n = 12).

Six months after the treatments, the chemical properties of soil 2 exhibited notable variations among the different treatment groups. However, in the soils treated with SBO, the reductions in both pH (measured in water) and KCl levels were particularly significant, surpassing the changes observed in soil 1. In contrast, EC, CEC, Organic Matter, C/N ratio, WSPs, and MBC all followed a similar increasing trend, as seen in soil 1 (Table 5).

When examining the biochemical data, akin to the findings in soil 1, the SBO-treated soil in soil 2 exhibited elevated levels of dehydrogenase fluorescein diacetate and beta-glucosidase activities, surpassing the other treatments, as previously observed. However, in the case of protease and urease activities, they displayed increases compared to control and NPK treatments, aligning with soil 1 (Table 6). Notably, they were found to be on par with the HM treatment, marking a departure from the results observed in soil 1.

Furthermore, catalase activity once again registered its lowest values in the SBO-treated soil, confirming the consistent pattern observed in soil 1.

The Pearson coefficient results for soil 1 indicated that all soil parameters were correlated with each other, albeit to varying degrees (Table 7). However, pH in H_2O showed a significant positive correlation only with WSPs, Catalase, and Total Nitrogen.

Table 7. Correlation matrix (Pearson (n)) of physical, chemical, and biochemical properties of soil 1 six months after treatments.

from\to	pH (H_2O)	pH (KCl)	EC	CEC	TOC %	TN %	C/N	SOM %	WSP	MBC	DHA	FDA	CAT	ßGLU	PRO	URE
pH (H_2O)	1	0.853	−0.581	−0.638	−0.974	0.724	−0.938	−0.974	0.845	−0.931	−0.806	−0.902	0.972	−0.344	−0.768	−0.792
pH (KCl)	0.853	1	−0.398	−0.649	−0.718	0.978	−0.965	−0.718	0.618	−0.904	−0.770	−0.771	0.735	−0.075	−0.902	−0.560
EC	−0.581	−0.398	1	0.923	0.666	−0.278	0.600	0.666	−0.922	0.738	0.891	0.865	−0.719	0.944	0.700	0.952
CEC	−0.638	−0.649	0.923	1	0.635	−0.586	0.768	0.635	−0.871	0.858	0.970	0.897	−0.698	0.761	0.905	0.884
TOC %	−0.974	−0.718	0.666	0.635	1	−0.556	0.857	1.000	−0.902	0.884	0.795	0.911	−0.996	0.488	0.679	0.862
TN %	0.724	0.978	−0.278	−0.586	−0.556	1	−0.894	−0.556	0.468	−0.814	−0.682	−0.647	0.578	0.055	−0.875	−0.411
C/N	−0.938	−0.965	0.600	0.768	0.857	−0.894	1	0.857	−0.802	0.983	0.888	0.909	−0.877	0.311	0.932	0.755
SOM %	−0.974	−0.718	0.666	0.635	1.000	−0.556	0.857	1	−0.902	0.884	0.795	0.911	−0.996	0.488	0.679	0.862
WSP	0.845	0.618	−0.922	−0.871	−0.902	0.468	−0.802	−0.902	1	−0.894	−0.935	−0.977	0.932	−0.791	−0.771	−0.996
MBC	−0.931	−0.904	0.738	0.858	0.884	−0.814	0.983	0.884	−0.894	1	0.954	0.969	−0.913	0.482	0.943	0.860
DHA	−0.806	−0.770	0.891	0.970	0.795	−0.682	0.888	0.795	−0.935	0.954	1	0.974	−0.843	0.691	0.940	0.928
FDA	−0.902	−0.771	0.865	0.897	0.911	−0.647	0.909	0.911	−0.977	0.969	0.974	1	−0.942	0.672	0.880	0.960
CAT	0.972	0.735	−0.719	−0.698	−0.996	0.578	−0.877	−0.996	0.932	−0.913	−0.843	−0.942	1	−0.537	−0.728	−0.896
ßGLU	−0.344	−0.075	0.944	0.761	0.488	0.055	0.311	0.488	−0.791	0.482	0.691	0.672	−0.537	1	0.428	0.843
PRO	−0.768	−0.902	0.700	0.905	0.679	−0.875	0.932	0.679	−0.771	0.943	0.940	0.880	−0.728	0.428	1	0.749
URE	−0.792	−0.560	0.952	0.884	0.862	−0.411	0.755	0.862	−0.996	0.860	0.928	0.960	−0.896	0.843	0.749	1

Red 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 green color and its gradations represent an inverse correlation, suggesting that the variables move in opposite directions.

In contrast, soil 2 exhibited lower correlations among its variables (Table 8). pH showed a positive correlation only with Catalase, and no significant positive correlations were observed among the other soil variables, except for TOC, which correlated with WSP, MBC, DHA, PRO, and MBC, which correlated positively with TOC and with all soil enzymes except for CAT) and URE. PCA analysis confirmed the data of Pearson coefficient evidencing differences between the relations of the two soils with the analyzed soil properties (Figures 2 and 3).

Table 8. Correlation matrix (Pearson (*n*)) of physical, chemical, and biochemical properties of soil 2 six months after treatment.

	pH (H_2O)	pH (KCl)	EC	CEC	TOC %	TN %	C/N	SOM %	WSP	MBC	DHA	FDA	CAT	ßGLU	PRO	URE
pH (H_2O)	1	0.360	−0.583	−0.966	−0.914	0.314	−0.765	−0.914	−0.961	−0.964	−0.824	−0.922	0.849	−0.923	−0.919	0.127
pH (KCl)	0.360	1	−0.924	−0.491	0.048	0.191	−0.173	0.048	−0.111	−0.514	−0.207	−0.349	0.472	−0.524	−0.315	−0.168
EC	−0.583	−0.924	1	0.737	0.216	−0.506	0.536	0.216	0.337	0.645	0.254	0.445	−0.495	0.606	0.415	−0.163
CEC	−0.966	−0.491	0.737	1	0.816	−0.511	0.853	0.816	0.867	0.918	0.666	0.814	−0.745	0.856	0.805	−0.286
TOC %	−0.914	0.048	0.216	0.816	1	−0.228	0.726	1.000	0.984	0.814	0.807	0.846	−0.718	0.769	0.858	−0.182
TN %	0.314	0.191	−0.506	−0.511	−0.228	1	−0.832	−0.228	−0.176	−0.131	0.278	0.075	−0.189	0.001	0.084	0.934
C/N	−0.765	−0.173	0.536	0.853	0.726	−0.832	1	0.726	0.693	0.592	0.285	0.458	−0.314	0.476	0.455	−0.735
SOM %	−0.914	0.048	0.216	0.816	1.000	−0.228	0.726	1	0.984	0.814	0.807	0.846	−0.718	0.769	0.858	−0.182
WSP	−0.961	−0.111	0.337	0.867	0.984	−0.176	0.693	0.984	1	0.903	0.878	0.925	−0.826	0.871	0.932	−0.073
MBC	−0.964	−0.514	0.645	0.918	0.814	−0.131	0.592	0.814	0.903	1	0.887	0.968	−0.948	0.991	0.961	0.109
DHA	−0.824	−0.207	0.254	0.666	0.807	0.278	0.285	0.807	0.878	0.887	1	0.974	−0.960	0.922	0.979	0.413
FDA	−0.922	−0.349	0.445	0.814	0.846	0.075	0.458	0.846	0.925	0.968	0.974	1	−0.978	0.980	0.999	0.261
CAT	0.849	0.472	−0.495	−0.745	−0.718	−0.189	−0.314	−0.718	−0.826	−0.948	−0.960	−0.978	1	−0.982	−0.973	−0.413
ßGLU	−0.923	−0.524	0.606	0.856	0.769	0.001	0.476	0.769	0.871	0.991	0.922	0.980	−0.982	1	0.973	0.245
PRO	−0.919	−0.315	0.415	0.805	0.858	0.084	0.455	0.858	0.932	0.961	0.979	0.999	−0.973	0.973	1	0.259
URE	0.127	−0.168	−0.163	−0.286	−0.182	0.934	−0.735	−0.182	−0.073	0.109	0.413	0.261	−0.413	0.245	0.259	1

Red 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, green color and its gradations represent an inverse correlation, suggesting that the variables move in opposite directions.

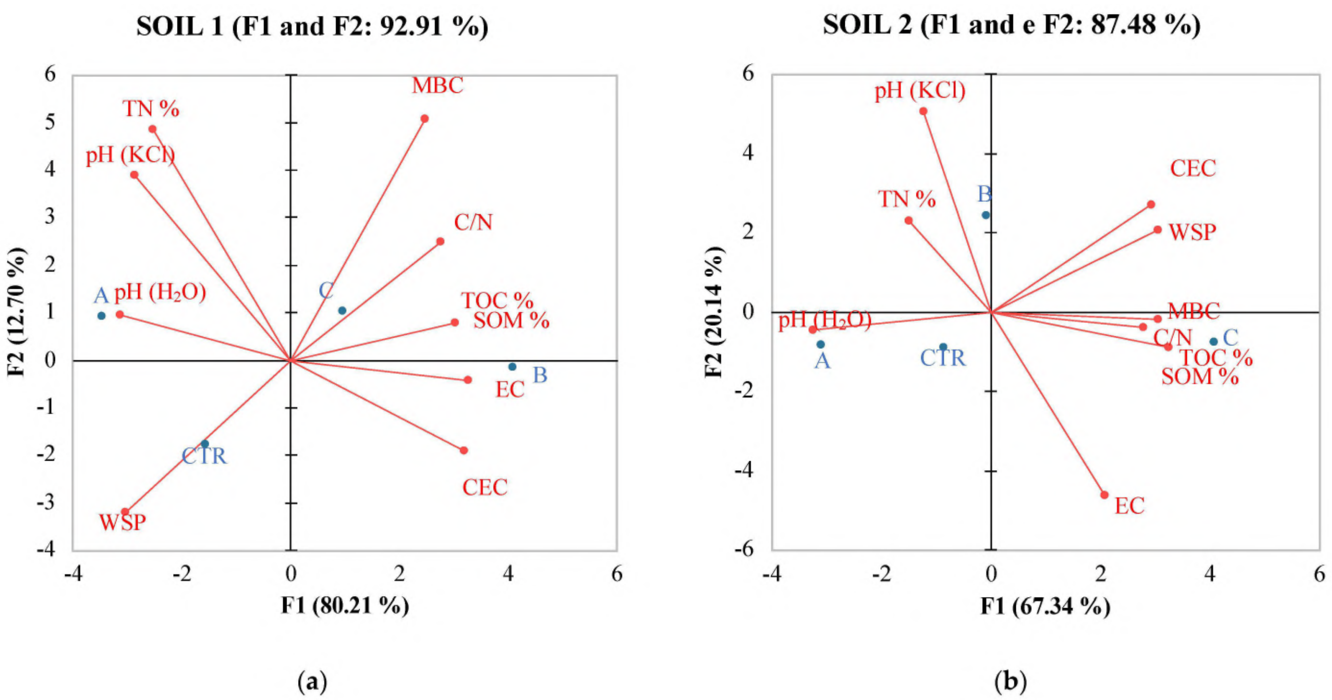
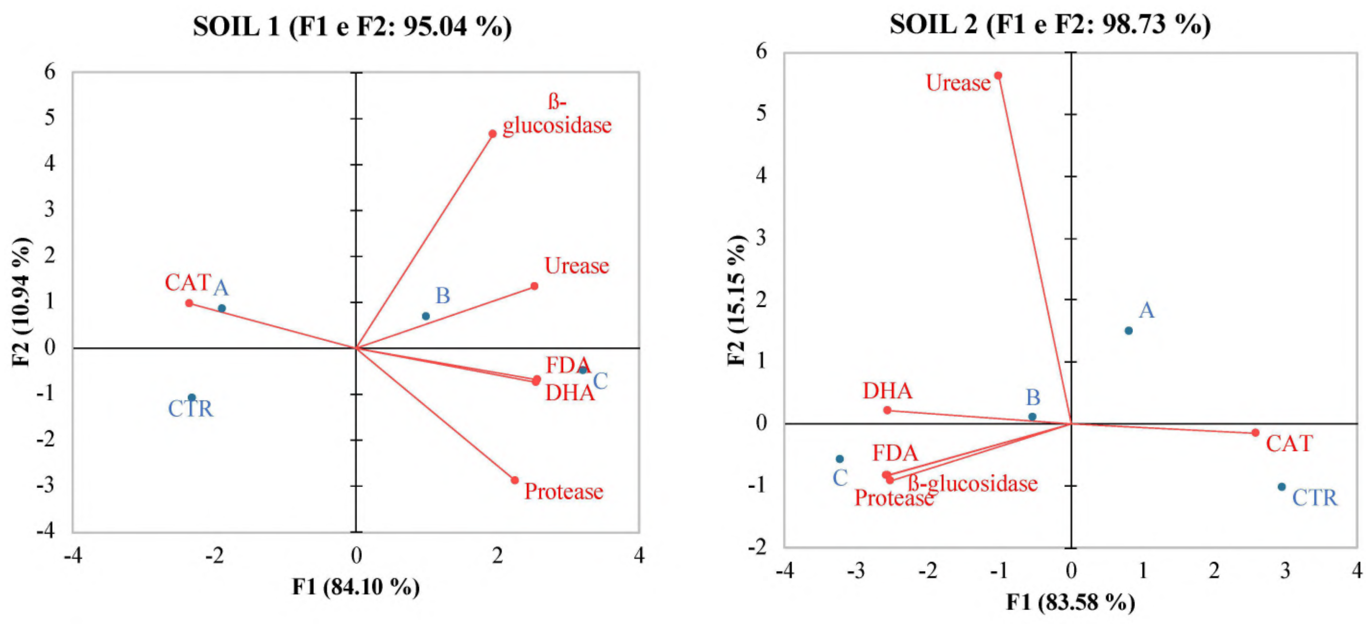


Figure 2. PCA of physical and chemical properties of soil 1 (**a**) and soil 2 (**b**) six months after treatments with the different fertilizers with CTR = Control, soil without fertilizer; A = nitrogen:phosphorous:potassium; B = horse manure; C = sulfur bentonite + orange residue.



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

Figure 3. PCA of enzymatic activities of soil 1 (**a**) and soil 2 (**b**) six months after treatments with the different fertilizers CTR = Control, soil without fertilizer; A = nitrogen–phosphorous–potassium; B = horse manure; C = sulfur bentonite + orange residue.

3.2. Environmental Impact

3.2.1. Carbon Footprint

The environmental analysis results, with a functional unit of 1 hectare and one ton of tomatoes considered for all impact categories, can be found in Tables 9 and 10. For Global Warming Potential (GWP 100a), Figure 4 reveals that among the four analyzed systems, System A has the highest impact at 26.04 kg CO_2 eq/t. This is followed by System B at 23.21 kg CO_2 eq/t and System CTR at 23.05 kg CO_2 eq/t. Notably, System C, which utilized Sulfur fertilizer bentonite, stands out as the most sustainable, with an impact value of 22.77 kg CO_2 eq/t.

Table 9. Environmental impacts per hectare of analyzed system CTR = Control, soil without fertilizer; A = nitrogen:phosphorous:potassium; B = horse manure; C = sulfur bentonite + orange residue.

Impact Categories	Unit	CTR	A	B	C
Abiotic depletion	kg Sb eq	0.01	0.02	0.01	0.01
Abiotic depletion (fossil fuels)	MJ	15,055.69	17,769.39	27,543.07	15,335.07
Global warming (GWP100a)	kg CO_2 eq	1083.46	1497.40	1346.10	1329.77
Ozone layer depletion (ODP)	kg CFC-11 eq	0.00	0.00	0.00	0.00
Human toxicity	kg 1.4-DB eq	494.38	650.97	530.92	508.03
Freshwater aquatic ecotox.	kg 1.4-DB eq	523.45	634.97	540.73	534.12
Marine aquatic ecotoxicity	kg 1.4-DB eq	629,032.75	803,512.81	678,553.69	648,589.00
Terrestrial ecotoxicity	kg 1.4-DB eq	1.02	1.57	1.24	1.09
Photochemical oxidation	kg C_2H_4 eq	0.19	0.22	0.29	0.19
Acidification	kg SO_2 eq	7.36	15.95	9.74	8.77
Eutrophication	kg PO_4—eq	1.96	4.16	2.28	2.50

Table 10. Environmental impacts for a ton of tomatoes for each analyzed system: CTR = Control, soil without fertilizer; A = nitrogen–phosphorous–potassium; B = horse manure; C = sulfur bentonite + orange residue.

Impact Categories	Unit	CTR	A	B	C
Abiotic depletion	kg Sb eq	0.00	0.00	0.00	0.00
Abiotic depletion (fossil fuels)	MJ	320.33	309.03	474.88	262.59
Global warming (GWP100a)	kg CO_2 eq	23.05	26.04	23.21	22.77
Ozone layer depletion (ODP)	kg CFC-11 eq	0.00	0.00	0.00	0.00
Human toxicity	kg 1.4-DB eq	10.52	11.32	9.15	8.70
Freshwater aquatic ecotox.	kg 1.4-DB eq	11.14	11.04	9.32	9.15
Marine aquatic ecotoxicity	kg 1.4-DB eq	13,383.68	13,974.14	11,699.20	11,105.98
Terrestrial ecotoxicity	kg 1.4-DB eq	0.02	0.03	0.02	0.02
Photochemical oxidation	kg C_2H_4 eq	0.00	0.00	0.01	0.00
Acidification	kg SO_2 eq	0.16	0.28	0.17	0.15
Eutrophication	kg PO_4—eq	0.04	0.07	0.04	0.04

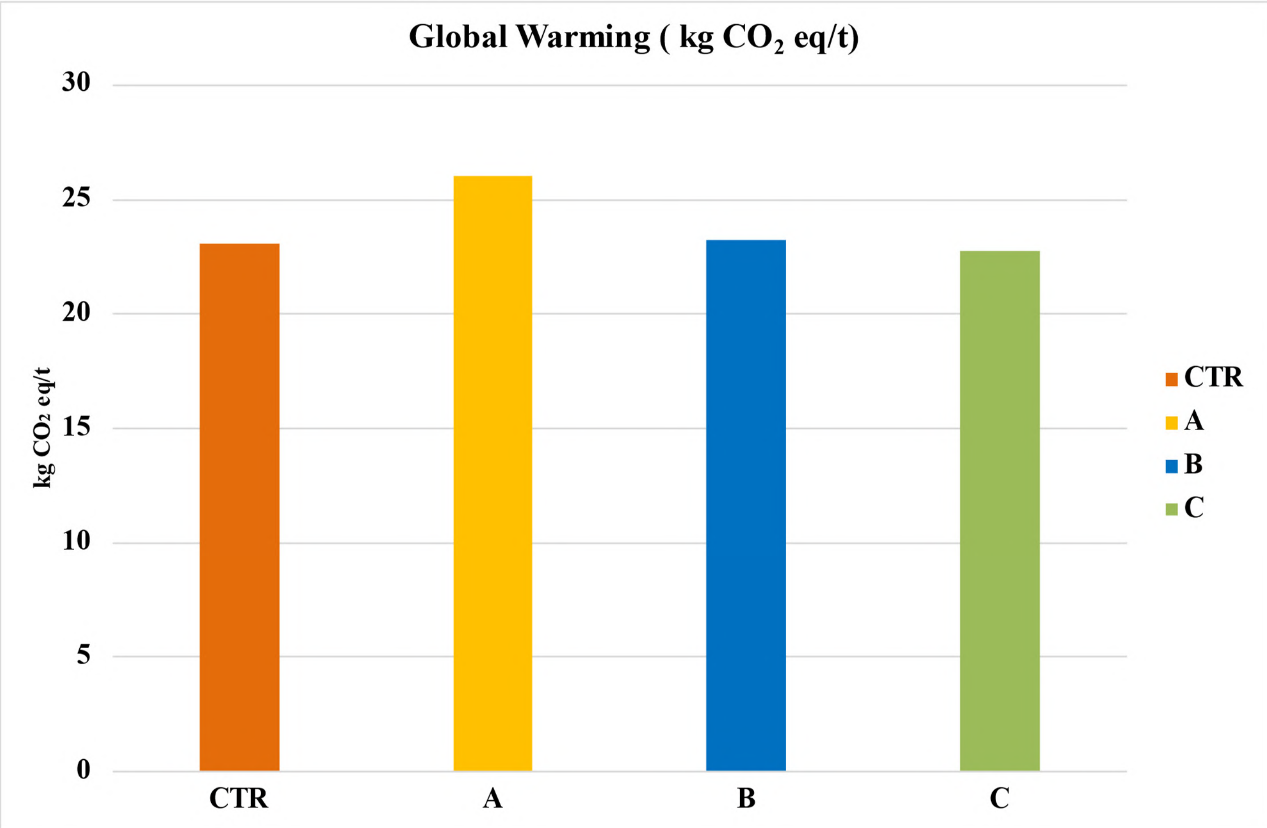


Figure 4. Global warming of the entire life cycle in terms of kg of CO_2 eq per ton of tomato. CTR = Control, soil without fertilizer; A = nitrogen:phosphorous:potassium; B = horse manure; C = sulfur bentonite + orange residue.

When broken down into individual phases, as seen in Figure 5, soil tillage emerges as the most impactful phase. This is followed by fertilization, registering an impact of 4.2 kg CO_2 eq/t when applied. The organic fertilization using horse manure (System B) has an impact of 4.5 kg CO_2 eq/t. Meanwhile, synthetic fertilization with NPK (System A) showcases the highest emissions among the systems, with values hitting 7.2 kg CO_2 eq/t.

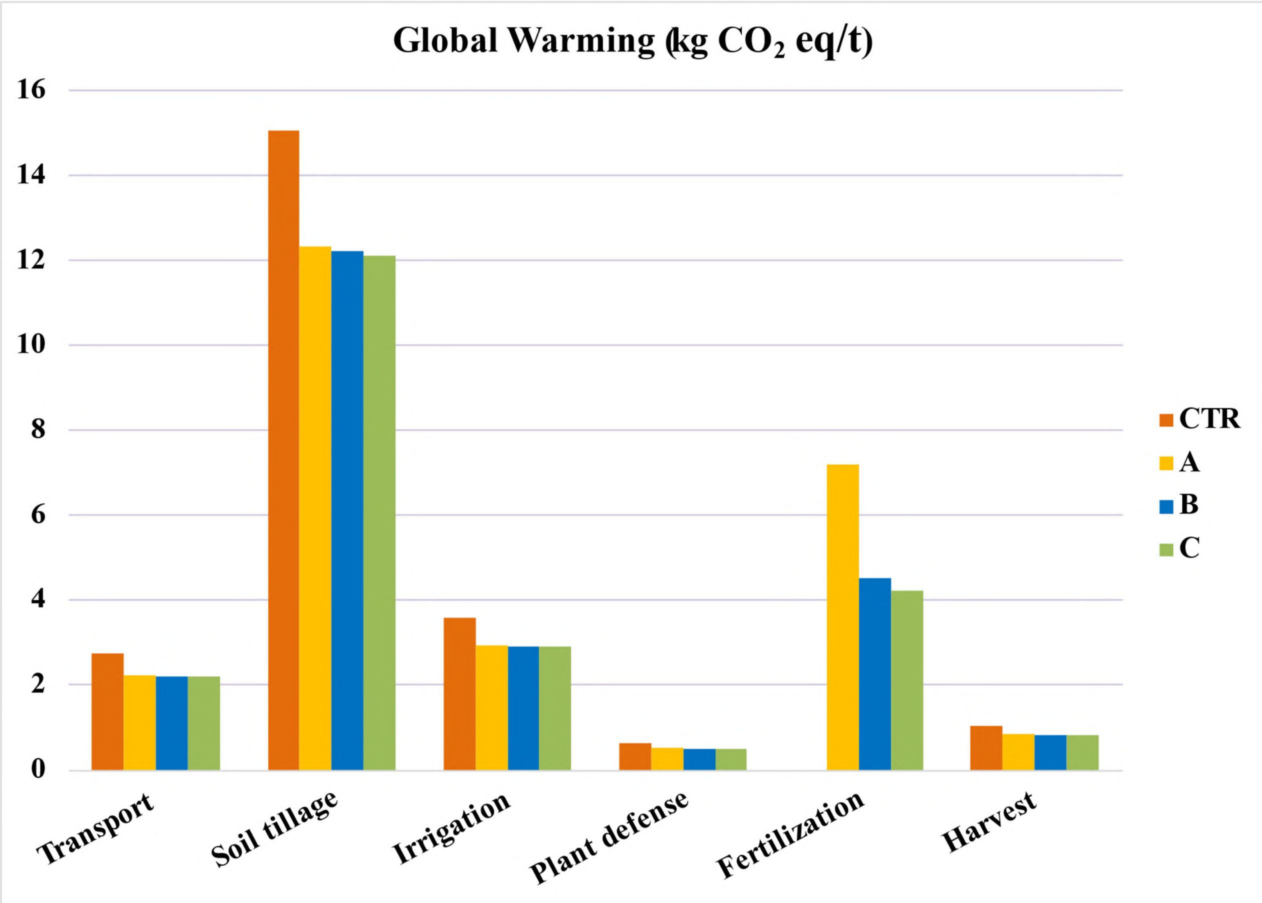


Figure 5. Global warming per phase of the production process. Values per ton of tomato. CTR = Control, soil without fertilizer; A = nitrogen:phosphorous:potassium; B = horse manure; C = sulfur bentonite + orange residue.

3.2.2. Water Footprint

The results of the WF are presented by breaking it down into its components (green, blue, and grey) and then as total water consumption. Table 11 gives the data on yields, green evapotranspiration (Et green), blue evapotranspiration (Et blue), direct and indirect fraction of water used for the different systems analyzed, with the WF expressed in m^3 per ton of tomato yield. Since all systems are located in Reggio Calabria (RC) and share identical climatic conditions (Table 4), green and blue evapotranspiration remains consistent across all systems, and differences in productivity result mainly from different fertilization approaches.

Table 11. WF green and Blue of the analyzed systems. CTR = Control, soil without fertilizer; A = nitrogen:phosphorous:potassium; B = horse manure; C = sulfur bentonite + orange residue.

Cultivation System	Yield (t/ha)	Et Green (m^3/ha)	Et Blue (m^3/ha)	Direct and Indirect Fraction (m^3/ha)	WF Green (m^3/t)	WF Blue (m^3/t)	WF Grey (m^3/t)
CTR	47.0	1983	1914	750.02	42.19	56.68	-
A	57.5	1983	1914	750.65	34.49	46.34	0.39
B	58.0	1983	1914	896.07	34.19	48.45	0.098
C	58.4	1983	1914	750.02	33.96	45.62	0.039

The system with the lowest WF is related to the use of sulfur-based bentonite fertilizers (A), which resulted in higher productivity. On the other hand, the control system (CTR) has the highest WF due to its lower production (Figure 6).

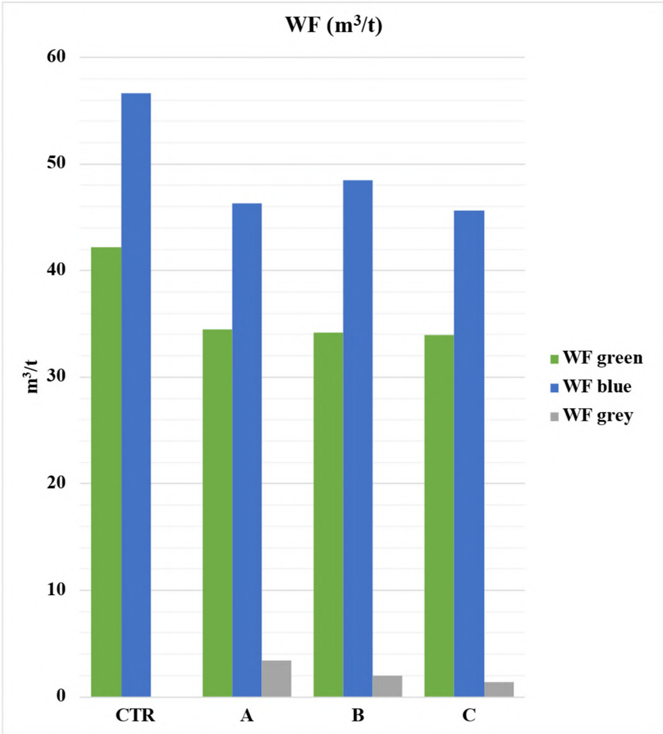


Figure 6. Total Water Footprint (m^3/t) broken down by its components (green, blue, grey) in the different cultivation systems. CTR = Control, soil without fertilizer; A = nitrogen–phosphorous–potassium; B = horse manure; C = sulfur bentonite + orange residue. Values are expressed in m^3/t of tomato.

4. Discussion

4.1. Effect of Different Fertilizers on Soil Quality

In both soil 1 and soil 2, the results indicated significant alterations in soil properties due to the application of SBO fertilizer. These changes encompassed shifts in soil pH, reductions in KCl levels, increased EC, and notable enhancements in organic matter content, CEC, and microbial biomass.

The observed decrease in phenol content and catalase activity in SBO-treated soils compared to other treatments suggested that SBO may have an impact on soil microorganisms and their metabolic activities. This is further supported by the results of the Pearson coefficient showing a positive correlation between MBC organic matter, pH in KCl, C/N ratio, WSP, FDA hydrolysis, dehydrogenase protease activity, and urease activities. These findings underscore the complex relationship between soil properties and microbial responses to fertilizer treatments. Our data agree with the findings of Arunrat et al. [55], showing that over a 5-year period, the application of fertilizer and tillage practices significantly contributed to an augmentation in the diversity and richness of soil bacteria. Regarding bacterial abundance, it was strongly influenced by organic matter (OM) and organic carbon (OC).

In soil 2, similar trends were observed with SBO treatment, leading to notable changes in soil properties and enzyme activities. However, the correlation pattern between MBC and other factors differed from that in soil 1, as shown in the correlation matrix data, highlighting the influence of soil characteristics on the effects of added fertilizers.

Furthermore, the reduced Catalase activity in SBO-treated soils suggested that this fertilizer does not induce stressful conditions in these soils, supporting its suitability for soil health and productivity. "The intricate and diverse relationship between catalase activity and microbial biomass, in the two soils, is underscored, influenced by factors such as soil pH, organic matter content, and temperature".

Principal Component Analysis (PCA) (Figure 2) results revealed multifaceted associations between SBO fertilizer and soil properties, both chemical and biochemical. These associations differed between soil 1 and soil 2, probably due to intrinsic dissimilarities in the soil conditions and microbial communities. Nonetheless, these findings provide valuable insights into the intricate interactions between fertilizers, soil characteristics, and microbial dynamics, which are essential for informed soil management practices and sustainable agriculture. Monitoring these parameters aids in assessing soil health, microbial activity, and nutrient cycling processes in differently-treated soil ecosystems, contributing to environmental management. The observed changes can positively affect tomato yield and quality. As already shown by Gao [56], tomato yield and quality were correlated to the amount of organic matter, which is known to play an important role in soil fertility and function. The trace elements in organic matter can meet the requirements of soil microorganisms, promote microbial activities, affect the soil–microorganism interaction, and consequently and indirectly influence tomato productivity. PCA analysis revealed, for soil 1, a positive correlation with SOM, MBC, TOC, and C/N ratio. This suggests a significant impact of SBO on soil chemical properties governing soil fertility. In contrast, for soil 2, an inverse correlation with these properties was observed. Turning to soil biochemical activity, in soil 1, the positioning in the II quadrant indicated an inverse correlation of SBO with fluorescein diacetate, dehydrogenase activity, and protease. This suggests a potential concentration threshold of SBO for this soil. Conversely, in soil 2, the positioning in the II quadrant revealed a positive correlation of SBO with FDA, protease, and beta-glucosidase.

Notably, HM and NPK did not show any significant relationship with the chemical and biochemical properties associated with soil fertility. In summary, these results underscore that SBO was the most effective fertilization strategy in both soils, and the variability in its effectiveness was linked to soil characteristics.

4.2. Environmental Impact

4.2.1. Carbon Footprint

The data offers a comparative understanding of the CF of various fertilization systems. System A's substantial impact underscores the environmental concerns surrounding synthetic fertilizers like NPK.

The significant reduction in CF when using sulfur fertilizer bentonite (System C) indicates its potential as an environmentally friendly alternative. The distinctions between the systems and the high emissions linked to synthetic fertilization emphasize the necessity for transitioning towards more sustainable farming practices.

The evident emissions related to soil tillage and fertilization emphasize the environmental implications of these farming activities. Furthermore, the disparity in emissions between organic and synthetic fertilization methods warrants attention.

System B's horse manure-based organic fertilization produces fewer emissions than the synthetic NPK method in System A, highlighting the environmental advantages of certain organic fertilizers. However, the fact that System C's Sulfur fertilizer bentonite outperforms even the organic method suggests there are innovative solutions that can further reduce agriculture's CF. Our results are in line with the findings of Wyngaard and Kissinger [57]. Theurl et al. [58], in a study comparing various tomato production systems in Austria, Spain, and Italy, showed that heating, packaging, and transport were the most important hot spots regarding GHG emissions associated with the different tomato supply chains and highlighted as the emissions from fertilizers, pesticides, soils, and infrastructures are relevant only in the case of intensive conventional production systems. Additionally, our results also agree with the findings of Toolkiattiwong et al. [59], indicating that more agricultural inputs, in terms of pesticides and fertilizers, augment the CFs and WFs and generate freshwater ecotoxicity.

4.2.2. Water Footprint

In the case of the cultivation system employing organic fertilizer (B), a higher impact is observed, mainly due to the direct fraction stemming from the water used in manure production. This indicates that organic fertilization may have implications for water use efficiency.

The results also revealed that the largest grey WF is associated with cultivation system A, which utilized synthetic NPK fertilizer. Conversely, due to the lower nitrogen content in sulfur bentonite fertilizer combined with orange residue, cultivation system C exhibits the lowest grey WF. This finding suggests that the choice of fertilizer can significantly impact the release of polluted water into the environment. Our results are in line with the findings of Evangelou et al. [60] and Wyngaard and Kissinger [57], who also showed how averages vary greatly depending on soil properties, local climatic conditions, and water management systems although no significant correlation the authors found with any individual soil property related to water retention. The high variability of WF values they found suggests the importance of considering water issues at the local scale level. As reported by Raluy et al. [61], WF results vary significantly within the same cultivars for the different local edaphoclimatic conditions and tree management models, as well as methodological choices adopted in the WF calculation.

When examining the Total WF, it becomes evident that the blue WF represents the largest percentage of WF when producing one ton of tomatoes for the various cultivation systems analyzed, followed by the green footprint. This highlights the importance of considering both surface and groundwater consumption in assessing the overall WF of agricultural practices.

While the grey WF constitutes only 1% of the total WF, it is a crucial indicator as it signifies the quantity of polluted water released into the environment. This underscores the need for sustainable fertilization practices to minimize environmental pollution.

Results evidenced that the impacts of the new fertilizer on the soil ecosystem varied in magnitude, consistently yielding positive effects on both soils. It can be confidently stated that the conversion of industrial and agricultural wastes into fertilizers holds the potential for economic and environmental benefits. These benefits arise from the reduced costs associated with waste disposal and the advantages to the soil resulting from a decrease in the use of mineral fertilizers, aligning with the principles and strategies of the circular economy. The results clearly demonstrated an enhancement in soil quality when utilizing sulfur-based pads, surpassing the performance of commonly employed organic and inorganic fertilizers. The continued reliance on the latter is discouraged in contemporary agriculture, particularly in the realm of organic farming.

In summary, the results emphasize the environmental benefits of System C, where tomato plants were fertilized with sulfur–bentonite combined with orange waste, as a more environmentally friendly choice in terms of WF. These findings contribute to the understanding of how fertilizer choices can influence water use efficiency and environmental impact in agriculture.

5. Conclusions

In summary, this fertilizer is environmentally friendly, and it plays a key role in fostering economic growth rooted in renewable resources. This responsible utilization of resources aligns seamlessly with the core tenets of a green economy, prioritizing the preservation and efficient use of resources. By minimizing its environmental footprint, this fertilizer actively contributes to the preservation of ecosystems and biodiversity—a cornerstone of a green economy. In doing so, it serves as a guardian of natural resources that form the bedrock of economic activities, particularly in agriculture. The sustainable production of this fertilizer has the potential to catalyze economic diversification by creating new markets and opportunities within the agricultural sector. This diversification can strengthen local economies, enhancing their resilience.

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Chapter 5 - Waste-Derived fertilizer acts as bio stimulant, boosting tomato quality and aroma

Mariateresa Russo[1], Rosa Di Sanzo[1], Federica Marra[1], Sonia Carabetta[1], Angela Maffia[1], Carmelo Mallamaci[1], Adele Muscolo[1*]

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

Correspondence: amuscolo@unirc.it

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Waste-Derived Fertilizer Acts as Biostimulant, Boosting Tomato Quality and Aroma

Mariateresa Russo, Rosa Di Sanzo, Federica Marra, Sonia Carabetta, Angela Maffia, Carmelo Mallamaci and Adele Muscolo *

Department of Agraria, "Mediterranea" University, Feo di Vito, 89122 Reggio Calabria, Italy; mariateresa.russo@unirc.it (M.R.); rosa.disanzo@unirc.it (R.D.S.); federica.marra@unirc.it (F.M.); sonia.carabetta@unirc.it (S.C.); angela.maffia@unirc.it (A.M.); carmelo.mallamaci@unirc.it (C.M.)

* Correspondence: amuscolo@unirc.it

Abstract: Tomato quality is intricately regulated by a combination of factors, including the presence of bioactive compounds referred to as secondary metabolites and various organoleptic characteristics. These attributes are notably influenced and harmonized by the specific growing conditions, with a particular emphasis on the type of fertilization employed. Traditionally, chemical fertilizers have been favored in crop cultivation due to their cost-effectiveness and ability to accelerate crop growth. However, in pursuit of sustainable and intelligent agricultural practices, there is a growing need for alternative fertilizers. In this context, the present study aimed to assess the impact of fertilizers derived from waste materials, specifically sulfur bentonite and orange residue (referred to as SB), on tomato quality. This assessment extended to examining qualitative and quantitative alterations in aroma-related volatile compounds and the antioxidant systems of tomatoes, in comparison to the conventional use of fertilizers such as horse manure (HM) and nitrogen, phosphorus, and potassium (NPK). The results obtained revealed distinct effects of different fertilizers on tomato quality. Notably, parameters such as TPRO (total protein), TCARB (total carbohydrate), LIC (lycopene content), TCAR (total carotenoid content), total phenols (TPHE), total flavonoids (TFLA), and aroma profiling exhibited significantly superior values in the group treated with sulfur bentonite (SB) fertilizer. These findings strongly suggest that the novel fertilizer functioned as a biostimulant, enhancing the nutraceutical and sensory attributes of tomatoes, with a pronounced impact on the synthesis of secondary metabolites and the aroma profile of the fruits.

Keywords: antioxidants; aroma profiling; biostimulant; fertilizer; phytochemicals; tomato

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1. Introduction

The tomato (*Solanum lycopersicum* L.) stands as one of the world's most beloved vegetables, belonging to the esteemed Solanaceae family. It claims the second spot in global vegetable consumption, following only potatoes and sweet potatoes. Moreover, it plays a significant role among canned vegetables, contributing substantially to the economic well-being of producer nations [1].

Tomatoes are celebrated for their culinary versatility and remarkable nutraceutical qualities. This adaptable fruit is savored in both its fresh and cooked forms by a diverse array of consumers. With only 30 calories per 100 g and a low fat content, it serves as a health-conscious choice. What distinguishes the tomato is its rich reservoir of antioxidants and its role as a potent source of essential vitamins (C and E), carotenoids (including lycopene and β-carotene), and a plethora of other phenolic compounds [2,3]. Owing to its substantial content of bioactive compounds, which remain largely intact during ripening and cooking and, in some cases, even become more pronounced, the tomato earns its reputation as a functional food [4].

In today's health-conscious society, consumers are increasingly drawn to vegetables replete with these bioactive compounds, celebrated for their positive impact on human health. Scientific evidence underscores their ability to safeguard cells from oxidative harm and act preventatively against the onset of degenerative conditions such as cancer, cardiovascular diseases, diabetes, Alzheimer's, and Parkinson's [5].

The global increase in tomato production can be attributed more to enhanced yields than to an expansion of cultivated land, primarily due to the overuse of fertilizers, especially chemical ones. Tomatoes grown in chemically over-fertilized soil are more susceptible to pest diseases, necessitating extensive pesticide use, which, in turn, has adverse effects on soil and human health. Crop quality, particularly in terms of nutritional value, has now assumed precedence over sheer productivity. Consequently, there is an urgent need to identify sustainable agricultural practices that can yield high-quality produce without compromising productivity.

Crop quality is intricately linked to the content of secondary metabolites, known as bioactive compounds, as well as organoleptic aspects that are influenced and balanced by growing conditions, particularly the type of fertilization employed [6]. Typically, chemical fertilizers are favored for their cost-effectiveness and rapid crop growth due to the readily available nutrients. Prior research has demonstrated the impact of different fertilization practices on the quality of various crops. Dumas et al. [7] revealed that the use of chemical fertilizers reduced the quantity of biocompounds with antioxidant properties. Young et al. [8] found that crops like cabbage, spinach, and peppers contained more antioxidants when grown with organic fertilizers than with chemical ones. Verma et al. [9] illustrated how bioaugmented compost improved antioxidant properties in tomatoes. Jin et al. [10] showed that reducing chemical fertilizers enhanced the quality of lettuce, and Moradzadeh et al. [11] indicated that the combined use of chemical and organic fertilizers improved agro-biochemical attributes of black cumin. Additionally, Akiyama et al. [12] demonstrated that tomatoes cultivated with organic fertilizers had higher nutritional values than those with chemical fertilizers.

The growth of tomatoes is significantly influenced by the presence of sulfur and sulfur-containing compounds, which serve vital roles as signaling molecules in normal metabolic processes and under stress conditions. A significant study by Silva et al. [13] highlighted that sulfur application led to increased tomato yield and fruit production. However, there is a dearth of comprehensive information regarding how sulfur fertilization may affect tomato quality, particularly concerning bioactive compounds and aromatic profiles. Furthermore, no previous research has explored the effects of sulfur fertilization when combined with organic components on tomato quality.

Given the aforementioned knowledge gap, this current study pursues two primary objectives:

To assess how the use of sulfur bentonite in conjunction with orange residue as a biostimulant influences tomato quality and its antioxidant systems, in comparison to the effects of horse manure and NPK fertilizer.

To investigate both the qualitative and quantitative alterations in the volatile compounds responsible for tomato aroma induced by sulfur bentonite, in comparison to horse manure and NPK fertilizer.

2. Materials and Methods

2.1. Site and Soil Details

The experimental sites were located in Motta San Giovanni, Loc. Liso, Italy, in soil classified as sandy loam (according to the Food and Agriculture Organization (FAO) soil classification system [14]), comprising 11.85% clay, 23.21% silt, and 64.94% sand. The soil exhibited a slightly alkaline pH and contained 3.09% organic matter and 0.17% nitrogen. Soil amendment was conducted in triplicate within the field. The soil was divided into 1 m square parcels. Each parcel received one of the following treatments: (1) sulfur bentonite–orange pads (SB) at a rate of 476 kg S ha^{-1}, (2) nitrogen–phosphorus–potassium (NPK, 20/10/10) at 170 kg ha^{-1}, (3) horse manure (HM) at 430 kg ha^{-1}, or (4) unfertilized soil used as the control (CTR). The experiment was arranged in a randomized complete block design with six parcels for each treatment, and the experiment was replicated for three consecutive years (2020/2021/2022). In each parcel, 3–4 tomato plants, variety Big Rio F1, per square meter were transplanted at the same growth stage, with uniform size, shape, and color. Regular watering was maintained to keep the water content at 70% of the field capacity in all parcels. Tomatoes treated with different fertilizers were harvested at the same stage of ripeness based on visual characteristics (uniform size, shape, and color). The results presented in the tables are the mean values from each parcel and across three consecutive years (n = 18).

2.2. Sample Preparation

A portion of the tomatoes treated with different fertilizers, all harvested at the same stage of ripeness (uniform size, shape, and color). The fruits were carefully mashed and homogenized and preserved in a −80 °C freezer before undergoing lyophilization, a process chosen to prevent any potential damage to the bioactive compounds and essential nutrients. The volatile fraction analyses were immediately conducted on freshly harvested fruits, which were cut into small pieces without grinding to prevent the development of secondary compounds.

2.3. Preparation of Ethanol and Water Extracts

The extracts were prepared following the method outlined by Kang [15], with minor adjustments, as detailed in Muscolo et al. [16].

2.4. Total Soluble Proteins

Soluble proteins, estimated as mg/g fresh weight (FW), were determined using the Bradford method, as reported in Muscolo et al. [16].

2.5. Total Available Carbohydrates

Total available carbohydrates were measured using the anthrone method with slight modifications, as described in Muscolo et al. [16].

2.6. Total Water-Soluble Phenols, Ascorbic Acid, Total Carotenoids, Total Flavonoids, and Vitamin E

The total water-soluble phenols were quantified using the Folin–Ciocalteu assay [17] with some minor adaptations, as reported in Muscolo et al. [16]. The absorbance was measured at 765 nm using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA).

Ascorbic acid was assessed in tomato powder (0.10 g) extracted with a solution of meta-phosphoric acid (3%)–acetic acid (7.98%) and centrifuged at 2365× g (4000 rpm) for 10 min. The measurement was conducted using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA), with absorbance at 525 nm, and ascorbic acid was detected in the supernatant using the Davies and Masten method [18].

Vitamin E was detected using the method of Prieto [19], with absorbance measured at 695 nm against the blank.

Flavonoids were estimated through the aluminum chloride colorimetric method of Djeridane et al. [20]. The absorbance was measured at 510 nm using a UV–Vis Agilent 8453 spectrophotometer (Agilent Technologies, CA, USA), and the results were expressed as rutin equivalents (mg/L) using a calibration curve.

Carotenoids (CAR) were extracted by grinding 50 mg of tomato in 25 mL of cold acetone, following the method outlined by Zhang et al. [21]. The absorbance of samples was measured at 537, 647, and 663 nm. The carotenoid content was expressed as mg g^{-1} of dry weight (dw).

2.7. Determination of Antioxidant Activities

The antioxidant activity against the DPPH radical (2,2-diphenyl-1-picryl-hydrazyl-hydrate) was determined using the method reported in Muscolo et al. [16]. The DPPH concentration in the cuvette was adjusted to yield absorbance values of ~1.0. The absorbance changes of the violet solution were recorded at 517 nm after 30 min of incubation at 37 °C. The inhibition (I%) of radical-scavenging activity was calculated using the formula I% = [(A0 − AS)/A0] × 100, where A0 is the absorbance of the control and AS is the absorbance of the sample after 30 min of incubation. The results were expressed as Trolox equivalents (TEs).

The total antioxidant capacity (TAC) was determined as per Muscolo et al. [16]. Sample absorbance was measured at 695 nm using a UV–visible spectrophotometer. Methanol (0.3 mL) was used as a blank in place of the extract. The antioxidant activity was expressed as μg of α-tocopherol per gram of dry weight (dw) based on a calibration curve.

The ABTS assay was conducted following the procedure described in Muscolo et al. [16]. Briefly, solutions containing 7 mmol L^{-1} ABTS•+ (final concentration) and 2.45 mmol L^{-1} ammonium persulfate (final concentration) in phosphate-buffered saline (PBS) were mixed and incubated in the dark at room temperature for 12–16 h. The absorbance of the samples was recorded at 734 nm using a UV–visible spectrophotometer. The percentage of radical-scavenging activity inhibition (I%) was calculated as I (%) = [(A0 − AS)/A0] × 100, where A0 represents the absorbance of the control and AS denotes the absorbance of the sample after 4 min of incubation. The results were expressed as μmol L^{-1} Trolox equivalents (TEs) based on a Trolox calibration curve.

2.8. Ultra-Fast Gas Chromatography Analysis

Ultra-fast gas chromatography (UFGC) analysis was carried out using the Heracles II instrument (Alpha MOS, Toulouse, France) equipped with an Odorscanner headspace autosampler (model HS 100, CTC Analytics, Zwingen, Switzerland) to automate sampling and injection. The Heracles II instrument featured two metal columns of different polarities working in parallel: a non-polar column (MXT-5: 5% diphenyl, 95% methylpolysiloxane) and a mid-polar column (MXT-1701: 14% cyanopropylphenyl, 86% methylpolysiloxane), both 10 mm in length and 0.18 mm in diameter. These columns were coupled to two flame ionization detectors (FID1 and FID2), enabling the simultaneous acquisition of two chromatograms for the identification of chemical compounds. The instrument was operated using AlphaSoft 2020 version 7.2.5 software, which included the AroChemBase module (Alpha MOS, Toulouse, France). The analysis of the volatile fraction was conducted on freshly harvested fruits, and samples were not subjected to grinding to prevent the development of secondary compounds. For each sample (6 × 3 replicates), approximately 2 mL of headspace was delivered at a rate of 125 μL/s from the autosampler to the injector, which was set to a temperature of 200 degrees Celsius. Further details of the UFGC settings can be found in Muscolo et al. [16].

2.9. Statistical Analysis

Statistical analyses were performed using one-way analysis of variance (ANOVA), and pairwise comparisons were conducted using Tukey's test. Statistically significant effects were determined at a significance level of $p \leq 0.01$. All data were analyzed using SYSTAT 13.0 for Windows (SPSS Inc., Chicago, IL, USA). Significant difference tests were employed to assess the impact of the three different fertilizers and the unfertilized soil on various measured parameters. Principal component analysis (PCA) was employed to explore relationships among different fertilizers and tomato parameters. PCA is an indispensable data analysis tool that aids in converting complex real-world datasets into manageable representative data. Additionally, PCA was applied to process the UFGC results, focusing on selecting features with the highest discriminatory power among samples. To visualize the results, the native UFGC program AlphaSoft 2020 version 7.2.5 (Alpha MOS, Toulouse, France) was utilized to generate a heat map for relative comparisons of each volatile compound.

3. Results and Discussion

Table 1 reveals that tomatoes treated with SB (sulfur and organic mix) exhibited significantly higher levels of total proteins (+40% compared to the control; +20% compared to NPK; +10% compared to HM) and carbohydrates (+30% compared to the control; +30% compared to NPK; +20% compared to HM). Additionally, lycopene content showed an impressive increase (+85% compared to the control; +36% compared to NPK; +15% compared to HM), as did carotenoid content (+40% compared to the control and NPK, and +15% compared to HM) in tomatoes cultivated with SB, surpassing HM, NPK, and control treatments (Table 1). This combination of heightened proteins and carbohydrates, along with a substantial increase in total carotenoids and lycopene in SB-treated tomatoes, enhances their nutraceutical value. These compounds collectively contribute to promoting human health and preventing various diseases, making SB-treated tomatoes a particularly nutritious choice.

Table 1. Water content (WC), dry weight (dw), fresh weight (fw), total proteins (TPRO, μg g^{-1} dw), total carbohydrates (TCARB, mg glucose g^{-1} dw), total phenols (TPHE, mg tannic acid g^{-1} dw), total flavonoids (TFLA, mg quercetin 100 g^{-1} dw), total carotenoids (CAR, mg 100 g^{-1} dw), licopene (LIC, mg 100 g^{-1} dw), total antioxidant capacity (TAC, mg alpha-tocopherol g^{-1} dw), 2,2′-diphenyl-1-picrylhydrazyl radical activity assay (DPPH•, % inhibition), vitamin A (VIT A, mg retinol 100 g^{-1} dw), vitamin C (VIT C, mg ascorbate 100 g^{-1} dw), and vitamin E (VIT E, mg alpha-tocopherol g^{-1} dw) in tomato grown in soils without fertilizer (control, CTR), with nitrogen/phosphorous/potassium (NPK), horse manure (HM), and sulfur bentonite with orange residue (SB). Data are the means ± standard errors of three replicates of three independent experiments (n = 18). * Different letters indicate significant differences per $p \leq 0.01$.

	CTR	NPK	HM	SB
WC	90.7 ^{a*}	89.2 ^{a}	90.7 ^{a}	89.7 ^{a}
Dry weight	9.3 ^{a}	10.8 ^{a}	9.3 ^{a}	10.3 ^{a}
Fresh weight	86 ^{b}	95 ^{a}	93 ^{a}	92 ^{a}
TPRO	1.2 ^{b}	1.3 ^{ab}	1.5 ^{a}	1.7 ^{a}
TCAR	17 ^{c}	16 ^{c}	21 ^{b}	24 ^{a}
LIC	14 ^{d}	19 ^{c}	23 ^{b}	26 ^{a}
TCARB	2.2 ^{b}	2.4 ^{ab}	2.6 ^{a}	2.8 ^{a}
TPHE	181.8 ^{b}	190.2 ^{b}	125.4 ^{c}	204.7 ^{a}
TFLA	361.8 ^{d}	389.9 ^{c}	511.3 ^{b}	533.3 ^{a}
VITA	132.5 ^{b}	137.3 ^{ab}	122.9 ^{c}	180.4 ^{a}
VITC	33 ^{c}	35 ^{b}	38 ^{b}	44 ^{a}
VITE	0.125 ^{a}	0.116 ^{a}	0.125 ^{a}	0.124 ^{a}
TAC	1.83 ^{b}	1.91 ^{b}	2.01 ^{b}	2.25 ^{a}
ABTS	0.018 ^{b}	0.029 ^{a}	0.032 ^{a}	0.035 ^{a}
DPPH%	43.9 ^{a}	36.6 ^{b}	45.5 ^{a}	37.2 ^{b}
DPPH	7.7 ^{b}	5.4 ^{c}	8.18 ^{a}	5.5 ^{c}

The remarkable increase in biomolecules observed in SB-treated tomatoes aligns with the findings of numerous other researchers who have emphasized the crucial role of sulfur (S) as a key nutrient for crop growth and development [22–24]. Sulfur is intricately involved in the synthesis of amino acids and proteins, making it indispensable for plant vitality. It is essential to note that a substantial proportion of soils, approximately 46%, are deficient in sulfur, and crops can only absorb a fraction of the S compared to nitrogen (N). This underscores the critical importance of sulfur fertilization, as it not only addresses this nutrient deficiency but also enhances the efficiency of nitrogen uptake, thereby maintaining a balanced nutrient profile [25–30].

The quality of tomatoes exhibited distinct responses to various fertilizers. As presented in Table 1, tomatoes treated with SB (sulfur and organic mix) outperformed other treatments, acting as a biostimulant and significantly elevating the levels of total phenols

(+10% compared to the control and NPK, and +50% compared to HM) and total flavonoids (+48% compared to the control, +38% compared to NPK, and +5% compared to HM). However, it is important to note that there were no significant differences observed in the vitamin E content among the differently treated tomatoes (Table 1). In contrast, a notable increase in the content of vitamin A (approximately 40% more than the control, NPK, and HM) and vitamin C (35% more than the control, 28% more than NPK, and 18% more than HM) was evident in tomatoes cultivated with SB fertilizer. This suggests that the combination of organic and elemental sulfur may provide a more effective nutritional boost compared to relying solely on either organic or inorganic fertilization. This enhanced nutrient availability can be attributed to the diverse range of micro and macro nutrients offered by this mixture, in contrast to mineral fertilizers, which primarily consist of only three major elements: nitrogen (N), phosphorus (P), and potassium (K), and organic fertilizers, which may lack sulfur.

Moreover, when assessing the total antioxidant capacity and ABTS levels, tomatoes fertilized with SB displayed the highest values (twice as high as the other treatments), while DPPH levels were similar to those of NPK-treated tomatoes, being the lowest among the treatments (Table 1). Two recent research articles [22,23] shed new light on the role of sulfur in the redox system. Sulfur emerges as a fundamental nutrient in the biosynthesis of secondary metabolites renowned for their high nutritional value. It has been convincingly demonstrated that sulfur exerts a positive influence on the accumulation of total phenols and flavonoids, compounds known for their potent antioxidant properties and remarkable nutraceutical value. Our data corroborate the findings of numerous other researchers, underscoring how sulfur fertilization not only augments total phenols and flavonoids in sulfur-loving crops such as garlic [30], cabbage [31], onion [32,33], and broccoli [25], but also in other species like artichoke [34] and tomato [35].

Total phenols and flavonoids possess significant antioxidant, anticancer, and antibacterial attributes. The above-mentioned compounds demonstrated efficacy as cardioprotective agents, anti-inflammatory substances, immune system boosters, and protective agents against UV radiation, thus exhibiting substantial potential for applications in the pharmaceutical and medical sectors [36–39].

The increase in total phenols and total flavonoids justified also the increase in antioxidant activities in SB-treated tomato.

The Pearson coefficient results revealed a significant positive correlation between total flavonoids, total antioxidant capacity (TAC), and, to a lesser extent, ABTS, while a negative correlation was observed with DPPH (Table 2). Total phenols did not show significant correlations with ABTS and TAC but exhibited a negative correlation with DPPH. In SB-treated tomatoes, the observed increase in carotenoids, known for their ability to prevent numerous chronic degenerative diseases through antioxidant action [40], confirmed the major role of total flavonoids, rather than total phenols, as antioxidants. Various studies have reported results showing a correlation between carotenoids, especially lycopene, and the mitigation of cancer and cardiac diseases [41,42].

Individual phenolic acids responded differently to the various fertilizations (Table 3). No significant differences were observed among the treatments for o-coumaric, 2,5 dihydroxybenzoic, and caffeic acids.

However, protocatechuic and syringic acids were only present in fertilized tomatoes compared to the control, with no differences noted between the various fertilizations. Trans-cinnamic acid was induced solely by the HM fertilizer, while trans-4-hydroxycinnamic acid exhibited the highest concentration in SB-treated tomatoes (+240% compared to the control; +420% compared to NPK; and +50% compared to HM) (Table 3).

These results suggest that the antioxidant activity found in SB-treated tomato could be related mainly and solely to trans-4-hydroxycinnamic acid.

Regarding the single flavonoids (Table 4), SB increased the synthesis of apigenin, tocopherol, vitexin, catechin, and naringin with respect to the control and the other fertilizers.

Table 2. Pearson correlation (r) between total proteins (TPRO, mg g^{-1} DW); total carotenoids (TCAR, µg 100 g^{-1} DW); lycopene (LIC, mg 100 g^{-1} DW); total carbohydrates (TCARB, mg glucose g^{-1} DW); total phenols (TPHE, µg GAE* g^{-1} DW); total flavonoids (TFLA, µg quercetin g^{-1} DW); vitamin A (VIT A, µg retinol 100 g^{-1} DW); vitamin C (VIT C, mg ascorbic acid g^{-1} DW.); vitamin E (VIT E, mg alpha-tocopherol 100 g^{-1} DW.); total anti-oxidant capacity (TAC, mg α-tocopherol/1100 g^{-1} d.w.); 2,2-diphenyl-1-picrylhydrazyl (DPPH, % inhibition); 1,1-diphenyl-2-picrylhydrazyl (DPPH radical, µM Trolox g^{-1} d.w.); and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS). Values in bold are different from 0 with a significance level alpha = 0.01.

Variables	TPRO	TCAR	LIC	TCARB	TPHE	TFLA	VITA	VITC	VITE	TAC	ABTS	DPPH %	DPPH
TPRO	**1**	**0.956**	**0.969**	**0.990**	0.044	**0.961**	0.700	**0.987**	0.293	**1**	0.882	−0.273	−0.273
TCAR	**0.956**	1	0.868	**0.908**	−0.028	**0.943**	0.653	**0.940**	0.559	**0.959**	0.717	−0.049	−0.048
LIC	**0.969**	0.868	1	**0.994**	−0.051	**0.953**	0.585	**0.936**	0.118	**0.969**	**0.969**	−0.315	−0.315
TCARB	**0.990**	**0.908**	**0.994**	**1**	0.014	**0.957**	0.656	**0.969**	0.178	**0.989**	**0.940**	−0.323	−0.324
TPHE	0.044	−0.028	−0.051	0.014	1	−0.232	0.735	0.199	−0.355	0.020	−0.057	−0.803	−0.801
TFLA	**0.961**	**0.943**	**0.953**	**0.957**	−0.232	1	0.481	**0.907**	0.400	**0.968**	0.865	−0.034	−0.035
VITA	0.700	0.653	0.585	0.656	0.735	0.481	1	0.804	0.050	0.684	0.494	−0.684	−0.683
VITC	**0.987**	**0.940**	**0.936**	**0.969**	0.199	**0.907**	0.804	1	0.255	**0.984**	0.843	−0.379	−0.378
VITE	0.293	0.559	0.118	0.178	−0.355	0.400	0.050	0.255	1	0.309	−0.113	0.694	0.695
TAC	**1**	**0.959**	**0.969**	**0.989**	0.020	**0.968**	0.684	**0.984**	0.309	1	0.880	−0.250	−0.249
ABTS	0.882	0.717	**0.969**	**0.940**	−0.057	0.865	0.494	0.843	−0.113	0.880	1	−0.417	−0.418
DPPH%	−0.273	−0.049	−0.315	−0.323	−0.803	−0.034	−0.684	−0.379	0.694	−0.250	−0.417	**1**	**1**
DPPH	−0.273	−0.048	−0.315	−0.324	−0.801	−0.035	−0.683	−0.378	0.695	−0.249	−0.418	**1**	**1**

Table 3. Single phenolic acids contained in differently cultivated tomato: without fertilizers (control, CTR) and with nitrogen/phosporous/potassium (NPK), horse manure (HM), and sulfur bentonite with orange residue (SB). Data are the means of three replicates of three independent experiments (n = 18). The experimental data are the mean of six replicates. Different letters in the same row indicate significant differences $p \leq 0.01$. nd = not detectable.

	CTR	NPK	HM	SB
Phenolic acids	mg/g SS	mg/g SS	mg/g SS	mg/g SS
Gallic	0.6 ^{a}	0.3 ^{b}	0.3 ^{b}	nd
Protocatechuic	nd	0.01 ^{a}	0.02 ^{a}	0.02 ^{a}
Syringic	nd	0.01 ^{a}	0.02 ^{a}	0.02 ^{a}
p-coumaric	nd	nd	0.01	nd
m-coumaric	4 ^{a}	0.6 ^{b}	nd	nd
o-coumaric	0.06 ^{a}	0.04 ^{a}	0.01 ^{a}	0.05 ^{a}
Trans-cinnamic	nd	nd	2.83 ^{a}	nd
3-hydroxycinnamic	nd	nd	nd	nd
Trans-4-hydroxycinnamic acid	0.46 ^{c}	0.3 ^{d}	1.00 ^{b}	1.58 ^{a}
Synaptic acid	0.02 ^{a}	0.04 ^{a}	0.04 ^{a}	nd
2,5 dihydroxy-benzoic acid	0.03 ^{a}	0.02 ^{a}	0.01 ^{a}	0.02 ^{a}
Caffeic acid	0.01 ^{a}	0.02 ^{a}	0.01 ^{a}	0.01 ^{a}
Chlorogenic acid	0.56 ^{b}	0.9 ^{a}	0.1 ^{a}	0.02 ^{c}
Ferulic acid	0.2 ^{b}	nd	0.34 ^{a}	nd

A recent manuscript [43] evidenced the important involvement of flavonoids in inflammatory response, highlighting their contribution to pathological pain by promoting plastic changes in the periphery and central nervous system, which in turn modify the neuronal phenotype and function. In particular, it was well demonstrated that these flavonoids diminished the neutrophil infiltration, had anti-inflammatory effect inhibiting cytokines, and antioxidant activity scavenging hydroxyl radicals; additionally, they also showed effects comparable to the corticoid prednisolone [35–38]. Pan et al. [39] evidenced that the quotidian consumption of flavonoid-rich foods was able to cause beneficial changes in the gut microbiota, diminishing the risk of cancer and normalizing vital functions at the cellular level [40]. In short, the data obtained evidenced that SB fertilization increased important phytochemical compounds in tomato, enhancing its nutraceutical value. Pearson correlation results between single phenolic acids and antioxidant activities evidenced a strong positive correlation between protocatechuic, syringic, and trans-4 hydroxycinnamic acid and ABTS and TAC (Figure 1).

Table 4. Single flavonoids contained in differently cultivated tomato: without fertilizers (control, CTR) and with nitrogen/phosporous/potassium (NPK), horse manure (HM), and sulfur bentonite with orange residue (SB). Data are the means of three replicates of three independent experiments (n = 18). The experimental data are the mean of six replicates. Different letters in the same row indicate significant differences $p \leq 0.01$. nd = not detectable.

	CTR	NPK	HM	SB
	mg/g SS	mg/g SS	mg/g SS	mg/g SS
Flavonoids				
Procyanidin 2	0.2 [a]	0.03 [b]	0.03 [b]	nd
Pelargonidine	0.05 [a]	nd	nd	nd
Cyanidine 3 O-glucoside	0.15 [a]	0.05 [b]	0.03 [b]	0.02 [b]
Catechin	0.08 [c]	0.15 [b]	0.3 [a]	0.3 [a]
Epicatechin	0.12 [a]	0.12 [a]	0.03 [b]	0.05 [b]
Delphinidin	0.54 [a]	0.52 [a]	0.1 [b]	0.1 [b]
Myricetin	1.16 [a]	1.42 [a]	nd	nd
Luteolin	0.04 [a]	0.03 [a]	0.02 [a]	0.03 [a]
Punicalagin	0.07 [a]	0.07 [a]	nd	nd
Naringin	nd	nd	nd	0.02 [a]
Quercetin	0.05 [a]	0.01 [a]	nd	0.02 [a]
Kaempferol	0.08 [c]	nd	2.1 [a]	0.16 [b]
Tocopherol	2.1 [b]	1.81 [c]	nd	2.99 [a]
Procyanidin 1	nd	0.16 [a]	nd	nd
Vicenin 2	0.01 [b]	0.08 [b]	0.3 [a]	0.08 [b]
Erythrocin	nd	0.05 [a]	nd	nd
Rutin	0.26 [c]	3 [a]	0.56 [b]	0.02 [d]

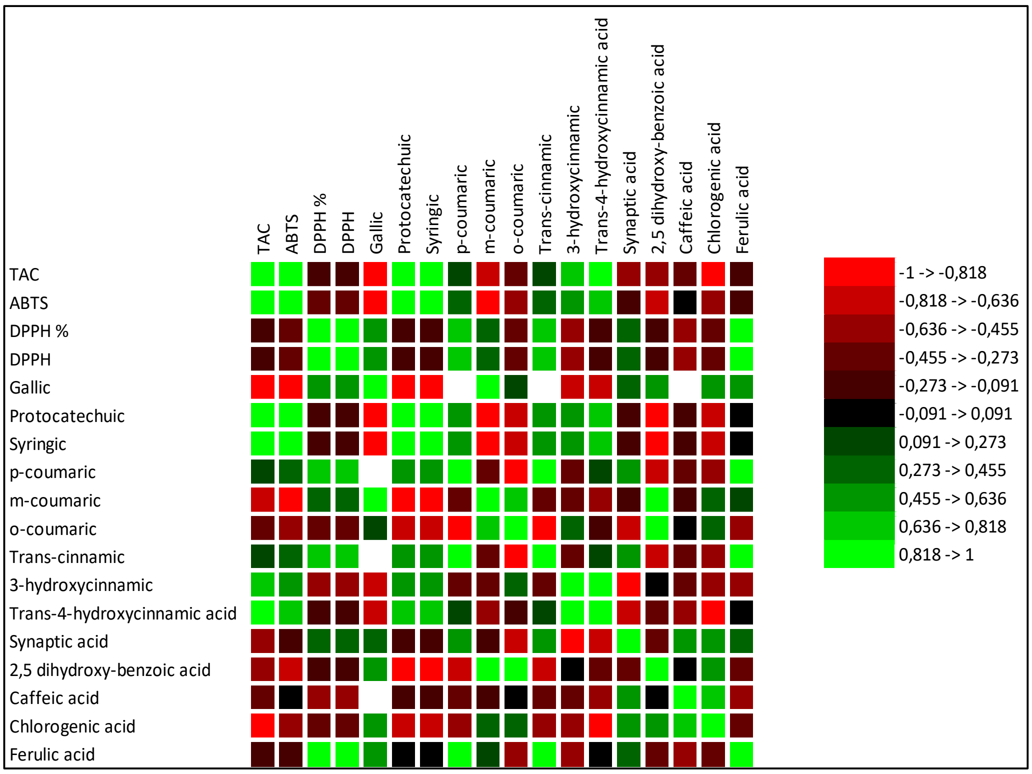

Figure 1. Pearson correlation coefficients (r) illustrating the relationships between individual phenolic acids and various antioxidant parameters, including total antioxidant capacity (TAC, mg α-tocopherol/100 g^{-1} d.w.), 2,2-diphenyl-1-picrylhydrazyl (DPPH, % inhibition), 1,1-diphenyl-2-picrylhydrazyl (DPPH radical, μM Trolox g^{-1} d.w.), and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS, % inhibition).

Conversely, ferulic acid correlated positively with DPPH. Regarding single flavonoids (Figure 2), only catechin, naringin, apigenin, and, at minor extent, vitexin positively and significantly correlated with TAC (Figure 2).

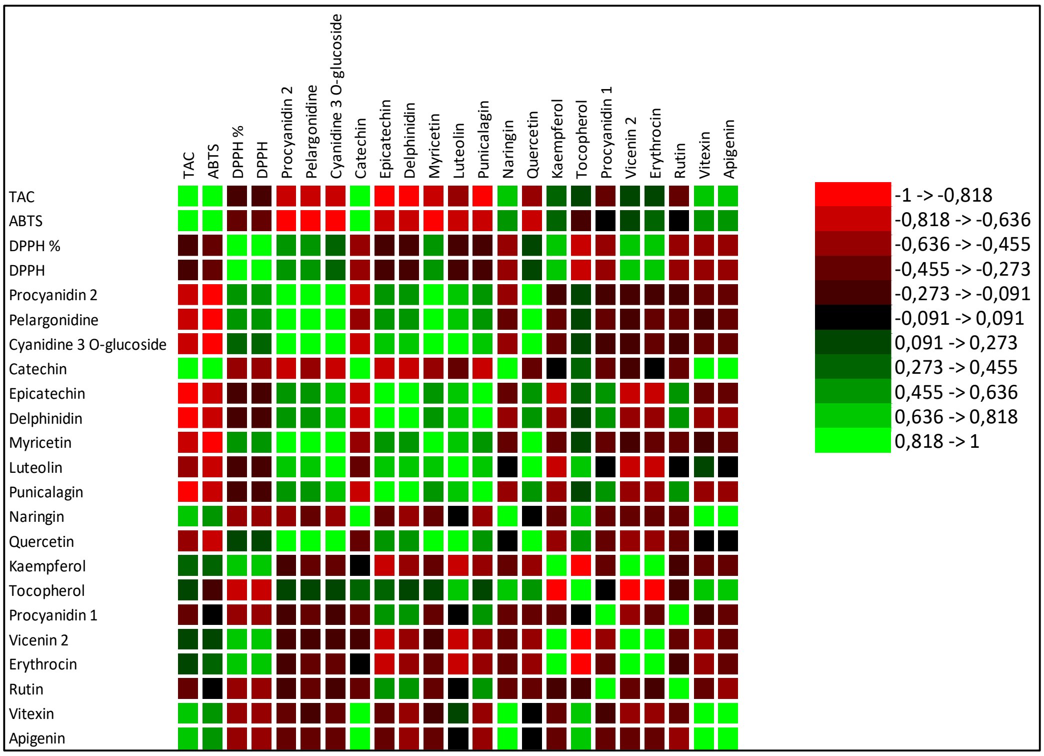

Figure 2. Pearson correlation coefficients (r) between individual flavonoids and various antioxidant parameters, including total antioxidant capacity (TAC, mg α-tocopherol*100 g^{-1} d.w.), 2,2-diphenyl-1-picrylhydrazyl (DPPH, % inhibition), 1,1-diphenyl-2-picrylhydrazyl (DPPH radical, μM Trolox g^{-1} d.w.), and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS, % inhibition).

ABTS correlated only with catechin; the other flavonoids were negatively or not correlated with ABTS, TAC, and DPPH activities. This result evidenced that, among the flavonoids, catechin correlated with both ABTS and TAC, activating anti-inflammatory and antioxidative responses. Considering that SB tomato contained the highest amount of trans-4 hydroxycinnamic acid, apigenin, catechin, naringin, and vitexin, its antioxidant value may be ascribed to these compounds that are positively and significantly correlated with TAC and ABTS. PCA analysis of primary and secondary metabolites evidenced positive effects of SB on vitamin A, ABTS, and total phenols (Figure 3). HM influenced the synthesis of primary metabolites, TAC, TCAR, VITE, and C (Figure 3). No positive effects were observed without fertilizations and in the presence of NPK (Figure 3).

PCA confirmed the positive correlation of SB with important single phenolic acids such as syringic, protocatechuic, and trans-4-hydroxycinnamic (Figure 4) with proven beneficial effects on human health for their antioxidant activities, as already highlighted by the Pearson correlation matrix. Single flavonoid synthesis was also affected by SB and, as reported in Figure 5, the flavonoids more affected by SB were catechin, apigenin, vitexin, and naringin—those that more correlated with the antioxidant activities.

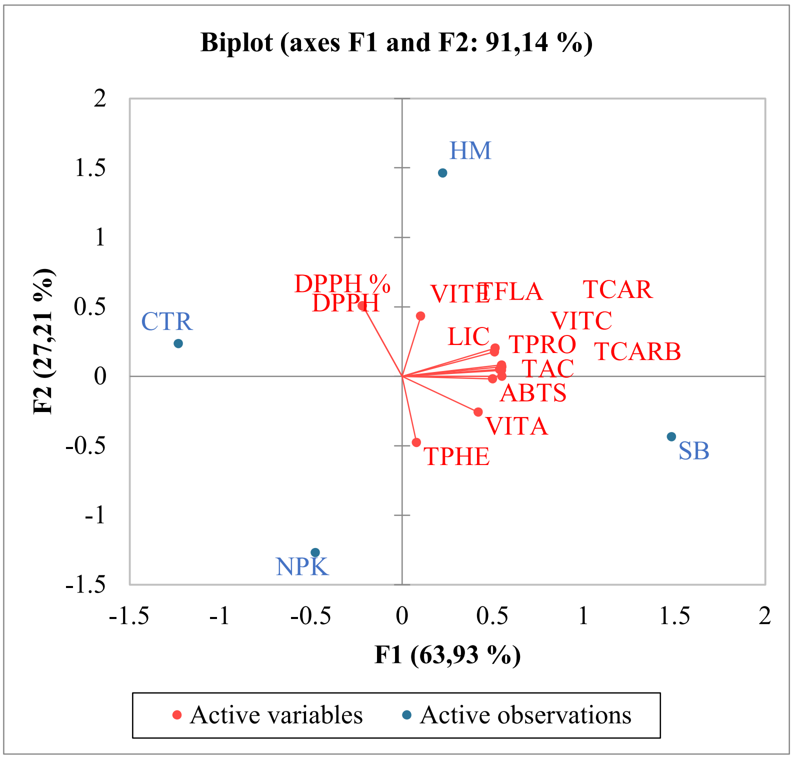

Figure 3. Principal component analysis (PCA) diagram depicting primary and secondary metabolites in tomatoes grown in distinct soil conditions: unfertilized soil (CTR) and soils enriched with various fertilizers, including nitrogen–phosphorus–potassium (NPK), horse manure (HM), and sulfur bentonite with orange residue (SB).

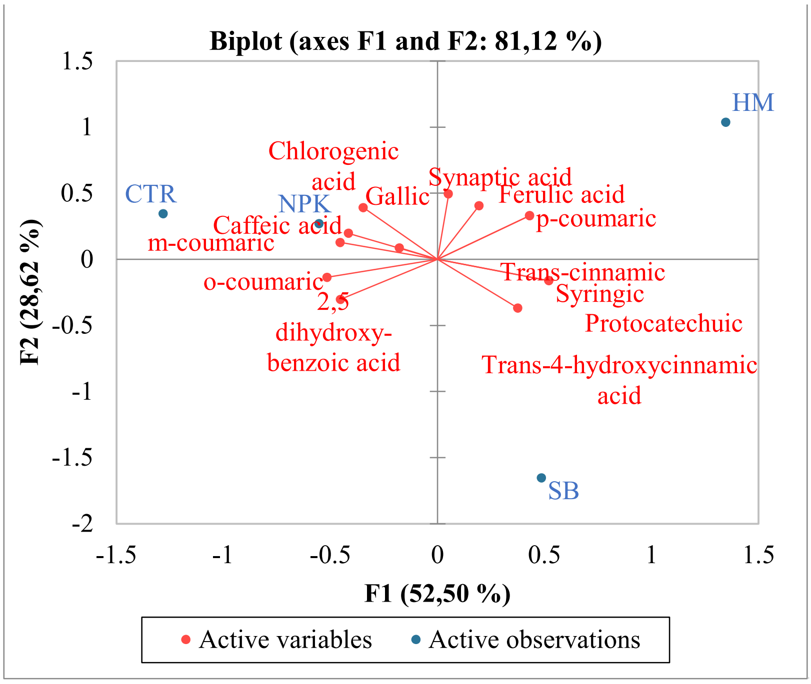

Figure 4. Principal component analysis (PCA) diagram representing individual phenolic acids in tomatoes cultivated in various soil conditions: unfertilized soil (CTR) and soils amended with different fertilizers, including nitrogen–phosphorus–potassium (NPK), horse manure (HM), and sulfur bentonite with orange residue (SB).

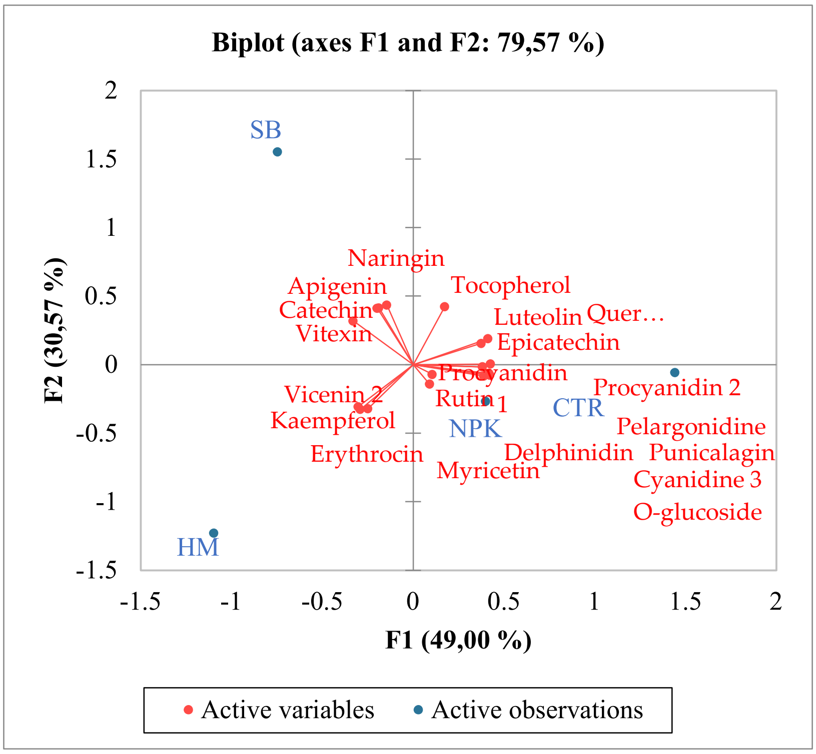

Figure 5. Principal component analysis (PCA) diagram illustrating the distribution of individual flavonoids in tomatoes cultivated under varied soil conditions: unfertilized soil (CTR) and soils enriched with different fertilizers including nitrogen–phosphorus–potassium (NPK), horse manure (HM), and sulfur bentonite with orange residue (SB).

In short, our results evidenced that SB was the fertilizer with biostimulant properties that influenced, in a prominent way, the quality of tomato fruits, increasing bioactive compounds with nutritional value and health benefit.

Regarding the aroma profiling, a comprehensive summary of discriminant chromatographic peaks and their associated sensory descriptors are reported in Table 5.

Table 5. Comprehensive summary of discriminant chromatographic peaks and their associated sensory descriptors (1-A: MTX5; 2-A: MTX 1701).

Retention Times	Name	Sensory Descriptors
13.56-1-A	acetaldehyde	Aldehydic; ethereal; fresh; fruity
49.70-1-A	3-heptanol	Green; herbaceous
61.40-1-A	ethyl hexanoate	Anise; apple; banana; berry; fruity; fruity(sweet); green; pineapple; strawberry; sweaty; sweet; unripe; waxy
66.63-1-A	(Z)-2-octenal	Earthy; fatty; fruity; green; leafy; walnut
10.68-2-A	unknown	
38.74-2-A	butane-2,3-dione	Butter; caramelized; chlorine; creamy; fruity; pineapple; pungent; spirit; strong; sweet
56.36-2-A	hexanal	Aldehydic; ethereal; fresh; fruity: green; herbaceous
90.80-2-A	1-nonanol	Dusty; fatty; floral; fresh; fruity; green; oily; orange; rose; wet

Forty-six (46) volatile compounds (Figure 6), were extrapolated from the chromatographic profiles. The volatile compounds identified in tomato were primarily aldehydes, alcohols, ketones, esters, organic acids, terpenes, and pyrazine compounds. Their relative intensities are shown as a heat map (Figure 7) .

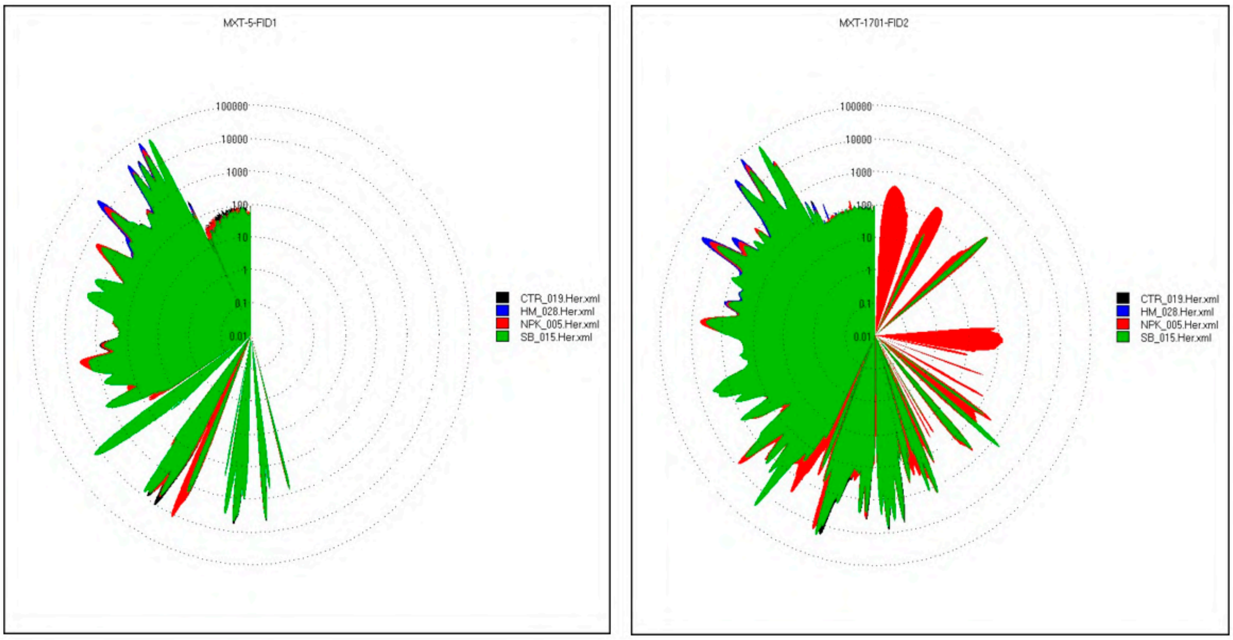

Figure 6. Odor maps or chemical fingerprints derived from UFGC analysis of tomato fruit samples cultivated in distinct soil conditions: unfertilized soil control (CONT), soil enriched with nitrogen–phosphorous–potassium (NPK), soil treated with sulfur bentonite and orange residue (SB), and soil amended with horse manure (HM).

Aldehydes were the main compounds in all samples with large intraclass variations, followed by alcohols, terpenes, and ketones. HM-treated tomato fruits had the highest concentration of aldehydes. The aromatic fraction of SB-treated tomato fruits was characterized by the highest percentage of aldehydes and a high concentration of esters and terpenes (+30%). Tomatoes fertilized with NPK were characterized by the highest percentage of aldehydes and a high concentration of both alcohols and terpenes (+20%). The pyrazine compounds were found only in tomato fruit fertilized with SB and NPK. The SB-treated tomato showed the highest percentage of both aldehydes and pyrazine compounds.

The four tomato samples were clearly distinguishable by their differences in the relative intensities of these factors. Tomato fruits treated with HM were the highest out of all samples in 1-propanol, 2-methyl-, and propanal (+20%). Tomato fruits fertilized with SB contained high levels of hexanal, followed by acetaldehyde and propanal, known to have ethereal and pungent characteristics (+22%). Tomato fruits fertilized with NPK showed relatively higher levels of propanal, 1-propanol, 2-methyl-, acetaldehyde, and ethyl hexanoate than the other treated tomatoes (+8%). Of these volatile compounds, the ethyl hexanoate is associated with fruity notes, and it plays a role in the discrimination of the NPK sample from the others (Figure 8). The tomato control showed relatively higher levels of acetaldehyde, propanal, hexanal and ethyl hexanoate than the others. Of the more than 400 volatile compounds found in ripe tomatoes, only 29 were present at concentrations greater than 1 ng L^{-1} or a one part per billion (ppb) [44]. Of these, approximately 16 had positive log odor unit values indicating a significant contribution to the tomato's aroma, including cis-3-hexenal, hexanal, 3-methylbutanal, trans-2-hexenal, trans-2-heptenal, 2-phenylacetaldehyde, β-ionone, 1-penten-3-one, β-damascenone, 6-methyl-5-hepten-2-one, cis-3-hexenol, 2-phenylethanol, 3-methylbutanol, 1-nitro-2-phenylethane, 2-isobutylthiazole, and methyl salicylate. Those volatiles that were slightly below the threshold contribute to the aromatic background [45]. The fingerprint, commonly used to distinguish food samples [46], showed evident differences between the three differently treated tomatoes compared to the control. The UFGC profiles were analyzed using PCA. In order to reduce the dataset measurements consisting of all the peak areas of each analyzed chromatogram, the most discriminant peak areas of specific compounds were extrapolated and then treated as an input dataset for PCA analysis [47]. In Figure 9, the radar chart evidenced the clear differences between the four samples analyzed. The differences between the chromatographic finger printings fully reflected the differences in the contents of some important components.

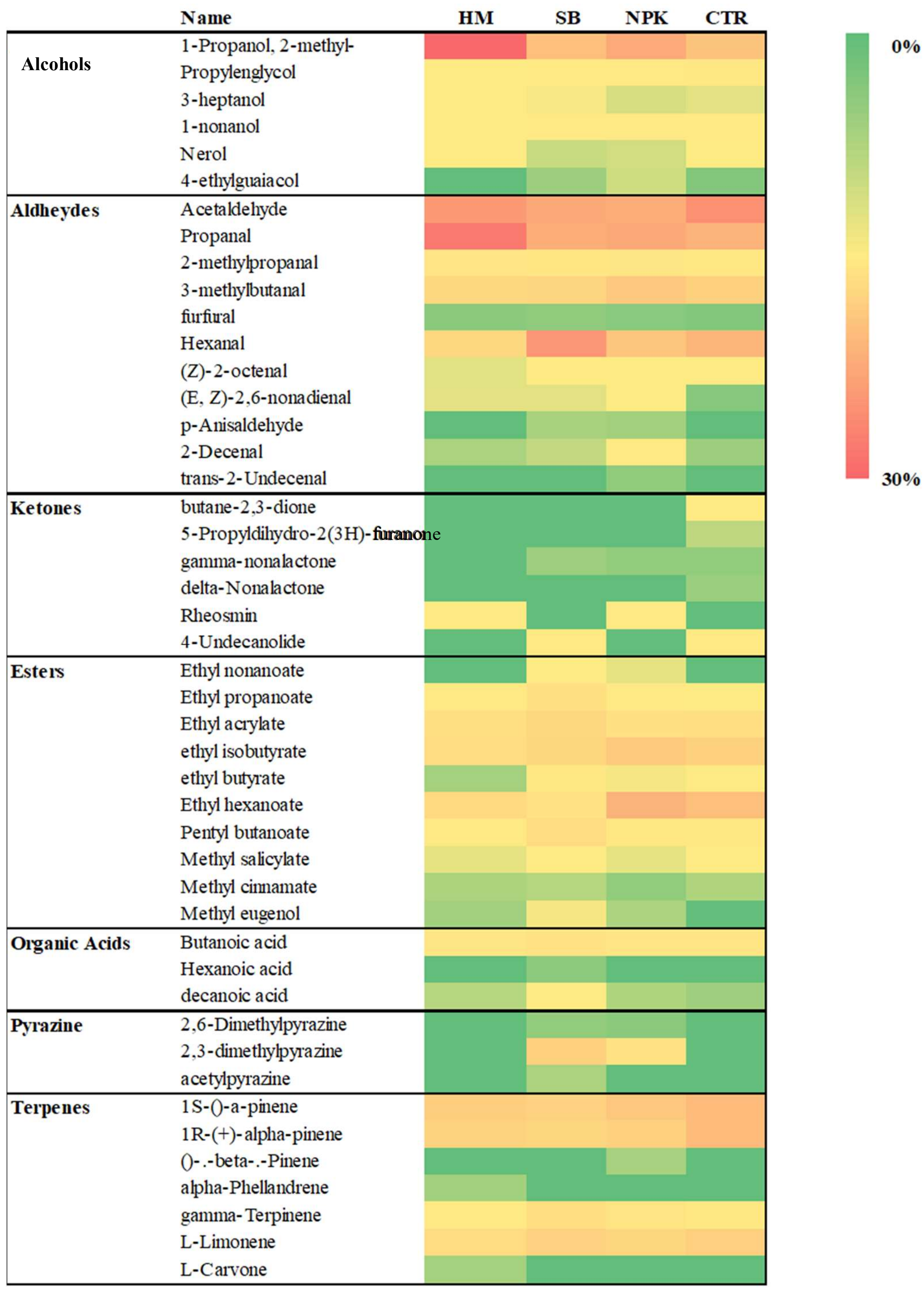

Figure 7. A heatmap displaying tomato fruit samples grown in different soil conditions: unfertilized soil control (CTR), soil with nitrogen–phosphorous–potassium fertilizer (NPK), soil treated with sulfur bentonite and orange residue (SB), and soil amended with horse manure (HM). The heatmap visualizes compound areas measured by UFGC, where green represents low peak areas and red indicates high peak areas, relative to each other.

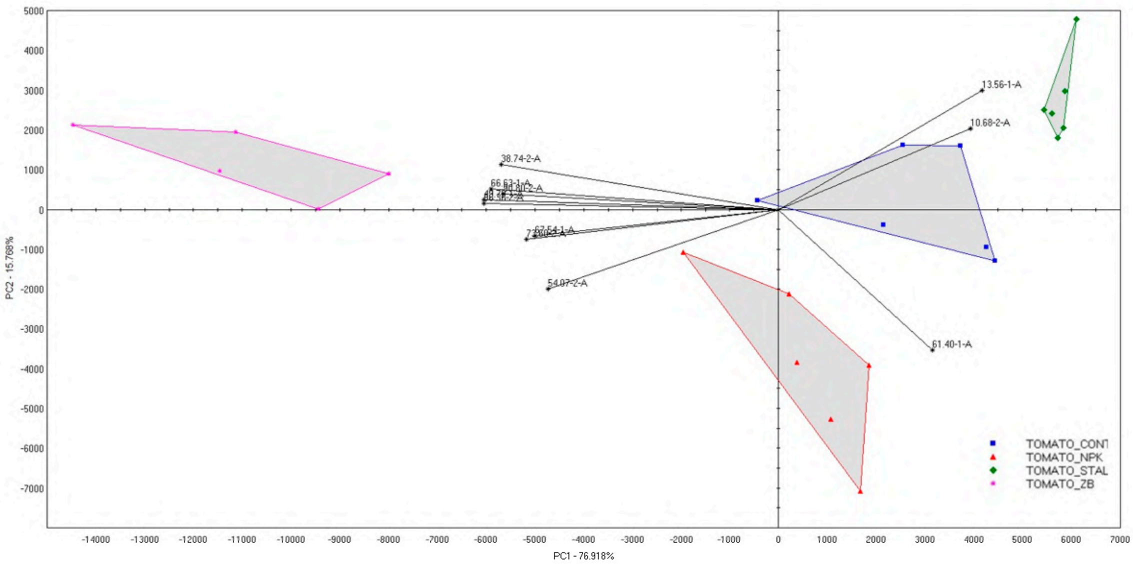

Figure 8. A PCA biplot illustrating the distribution of tomato samples cultivated in different soil conditions: unfertilized soil control (CTR), soil enriched with nitrogen–phosphorous–potassium (NPK), soil treated with sulfur bentonite and orange residue (SB), and soil amended with horse manure (HM). This biplot highlights the discrimination of odorous compounds, including acetaldehyde (13,56-1-A), 3-heptanol (49,70-1-A), ethyl hexanoate (61,40-1-A), (Z)-2-octanal (66,63-1-A), an unknown compound (10,68-2-A), butane-2,3-dione (38,74-2-A), hexanal (56,36-2-A), and 1-nonanol (90,80-2-A).

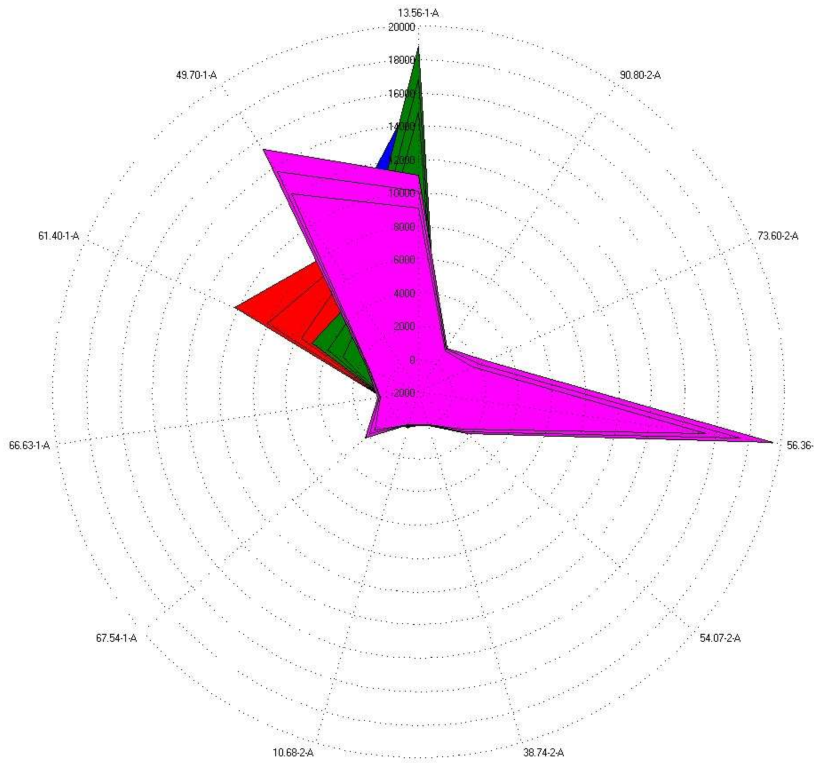

Figure 9. Radar chart illustrating discriminant peaks of tomato samples grown in different soil conditions: unfertilized soil control (CTR), nitrogen–phosphorous–potassium fertilized soil (NPK), soil with sulfur bentonite mixed with orange residue (SB), and soil with horse manure (HM). The chart discriminates based on the following odorous compounds: acetaldehyde (13,56-1-A), 3-heptanol (49,70-1-A), ethyl hexanoate (61,40-1-A), (Z)-2-octanal (66,63-1-A), unknown compound (10,68-2-A), butane-2,3-dione (38,74-2-A), hexanal (56,36-2-A), and 1-nonanol (90,80-2-A).

Acetaldehyde, (Z)-2-octenal, hexanal, 1-nonanol, and butane-2,3-dione were responsible for the fresh and fruity flavor. These compounds also promote the fresh feeling of the fruit and participate in the formation of the sweet character [48]. Fruity, green, and unripe flavor is related to the ethyl hexanoate and 3-heptanol. The heat map (Figure 10) showed the differences in the aroma profile of the differently treated samples. C6 volatile compounds, including hexanal, trans-2-hexene, cis-3-hexene, and corresponding alcohols, were among the most abundant volatile compounds in tomatoes, giving "green" and "grassy" notes to the fruit [49]. The highest value of hexanal was found in tomato fruits treated with SB. The PCA analysis (Figure 8) showed the first component discriminated only the samples SB and HM, while NPK and CTR were discriminated by the second ones. The SB group was absolutely different from the other groups. Odorous compounds, acetaldehyde (3,56-1-A) and an unknown (10,68-2-A), were characteristics of the HM-treated tomato and CTR groups; 3-heptanol (49,70-1-A), ethyl hexanoate (61,40-1-A), (Z)-2-octanal (66,63-1-A), butane-2, 3-dione (38,74-2-A), hexanal (56,36-2-A), and 1-nonanol (90,80-2-A) were characteristics of the SB group; and ethyl hexanoate (61,40-1-A) was characteristic of the NPK group.

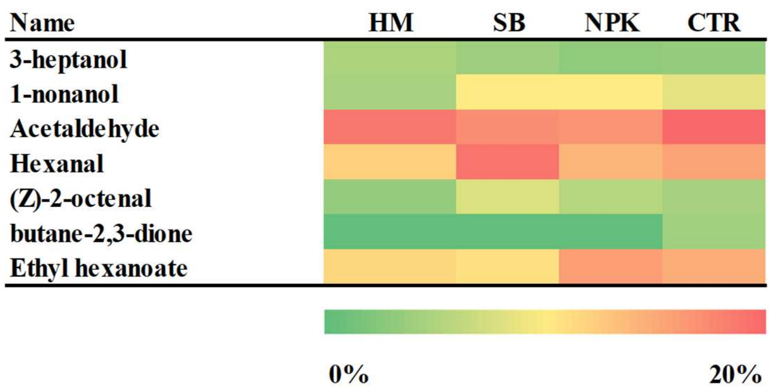

Figure 10. A heatmap displaying discriminant chromatographic peaks of tomato fruit samples grown under various soil conditions: unfertilized soil control (CTR), soil with nitrogen–phosphorous–potassium fertilizer (NPK), soil enriched with sulfur bentonite and orange residue (SB), and soil amended with horse manure (HM). The heatmap represents the peak areas measured by UFGC, with green indicating low peak area and red indicating high peak area, respectively.

In summary, the analysis of odor profiles revealed that SB-treated tomatoes had the highest percentage of C6 aldehydes, such as hexanal, often referred to as a "green" compound. Hexanal imparts a fresh, green character to the tomato aroma and can induce the activation of defense genes that enhance tolerance against fungi even at relatively low concentrations [50–54].

4. Conclusions

The fertilization of crops, either chemical or organic, has been recommended, up until now, to improve soil productivity and compensate for the lack of nutrients. As this study has shown, fertilizers from agro-industrial wastes containing both single nutrients and organic components can be used instead as improvers of soil but also as improvers of crop quality. The aromatic profiles of treated tomato, in good agreement with the secondary metabolites, have been heavily modified in intensity and composition using SB, which differently influenced the production of bioactive compounds, increasing the bioactive compounds' antioxidant activity and the main compounds responsible for the best characteristics of tomato flavor. Taken together, these results highlight that fertilizers produced by wastes can be used as biostimulants to strengthen bioactive compounds in fruits, providing a new strategy to ameliorate the nutraceutical power and profitability of crops with prominent results on the bio and green economy.

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Chapter 6 - 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], Muhammed Iftikhar Hussain[3], Adele Muscolo[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 Poalo 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

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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

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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:

$$\text{Organic matter loss} = (\text{initial mass of carbon} - \text{final mass of carbon}/\text{initial mass of carbon}) \times 100$$

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.

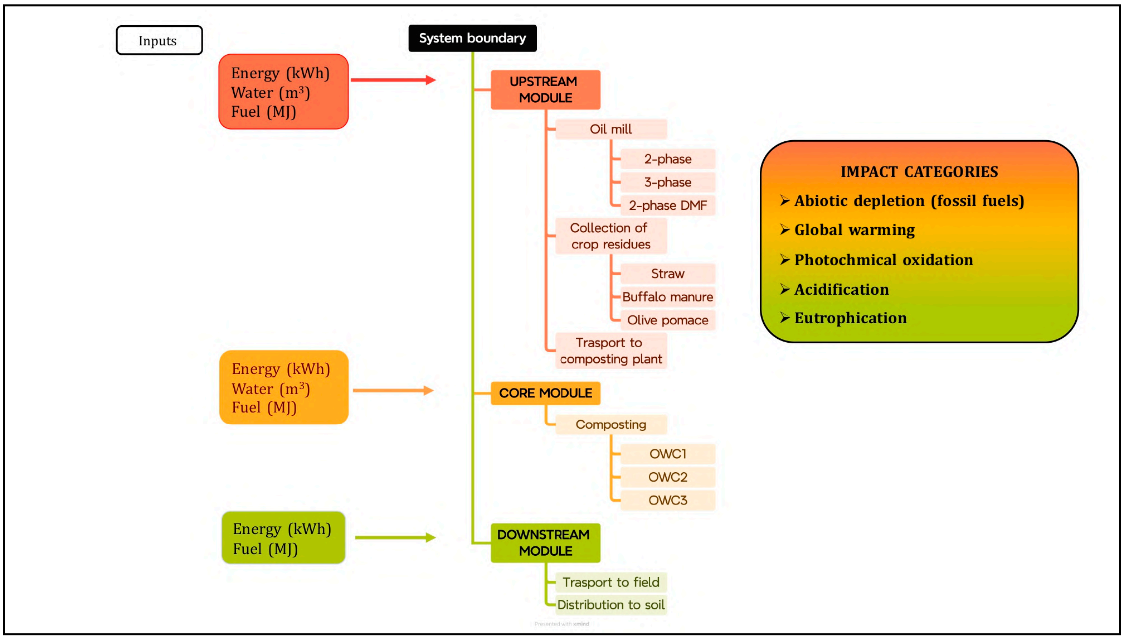


Figure 1. System boundaries divided by upstream, core, and downstream modules for assessing the environmental impacts shown in the figure of OWC1, OWC2 and OWC3.

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*} ± 0.11	5.84 ^{b} ± 0.28	2.12 ^{b} ± 0.35	42.6 ^{c} ± 0.34	48.05 ^{b} ± 0.28	1.67 ^{b} ± 0.001	28.74 ^{b} ± 0.40	1.60 ^{c} ± 0.06	90.10 ^{a} ± 1.68
OWC2	5.34 ^{c} ± 0.35	4.88 ^{c} ± 0.19	11.83 ^{a} ± 0.80	58.5 ^{b} ± 0.55	42.44 ^{b} ± 0.49	1.64 ^{b} ± 0.003	25.87 ^{b} ± 0.51	11.00 ^{a} ± 0.41	85.00 ^{b} ± 1.36
OWC3	7.80 ^{a} ± 0.17	7.10 ^{a} ± 0.11	2.60 ^{b} ± 0.74	58.3 ^{a} ± 0.76	62.61 ^{a} ± 0.56	1.96 ^{a} ± 0.004	31.90 ^{a} ± 0.55	6.08 ^{b} ± 0.15	81.00 ^{c} ± 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*} ± 0.05	0.17 ^{b} ± 0.01	17.90 ^{b} ± 0.02	1.40 ^{a} ± 0.02	0.29 ^{b} ± 0.04	0.80 ^{b} ± 0.11	0.01 ^{a} ± 0.004	0.05 ^{a} ± 0.010	0.50 ^{b} ± 0.04	0.39 ^{b} ± 0.03
OWC2	0.79 ^{b} ± 0.04	0.28 ^{a} ± 0.03	20.29 ^{a} ± 0.01	1.19 ^{b} ± 0.04	0.56 ^{a} ± 0.01	3.77 ^{a} ± 0.47	0.01 ^{a} ± 0.003	0.01 ^{a} ± 0.002	2.12 ^{a} ± 0.21	1.21 ^{a} ± 0.15
OWC3	1.15 ^{a} ± 0.02	0.11 ^{c} ± 0.01	18.43 ^{b} ± 0.01	1.32 ^{a} ± 0.12	0.43 ^{a} ± 0.07	2.65 ^{a} ± 0.02	0.02 ^{a} ± 0.14	0.04 ^{a} ± 0.002	1.15 ^{a} ± 0.03	1.12 ^{a} ± 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*} ± 0.10	81.12 ^{a} ± 2.16	39.22 ^{b} ± 1.16	22.95 ^{b} ± 0.86
OWC2	2.67 ^{a} ± 0.11	16.55 ^{c} ± 5.21	80.12 ^{b} ± 3.57	25.13 ^{b} ± 1.03
OWC3	1.60 ^{b} ± 0.11	65.60 ^{b} ± 2.21	89.31 ^{a} ± 4.11	28.81 ^{a} ± 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.

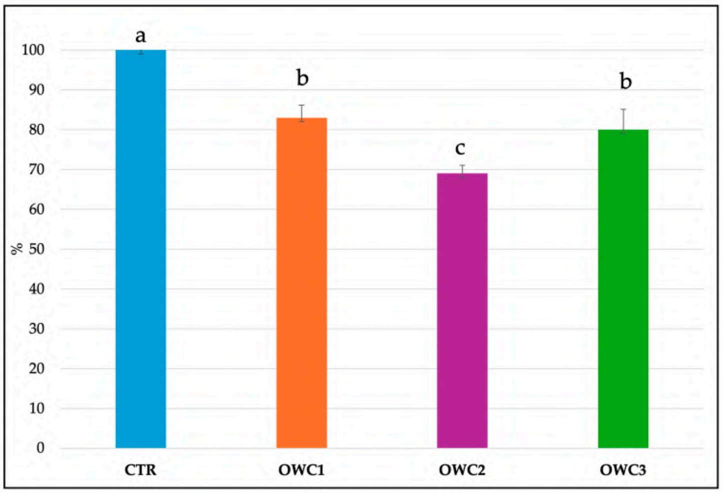


Figure 2. Global germination index of *Cucumis sativus* L. (%) in the presence of selected concentrations of the different composts. Data are the mean of 3 replications ± standard deviation. Different letters indicate significant differences (Turkey's test $p < 0.05$).

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).

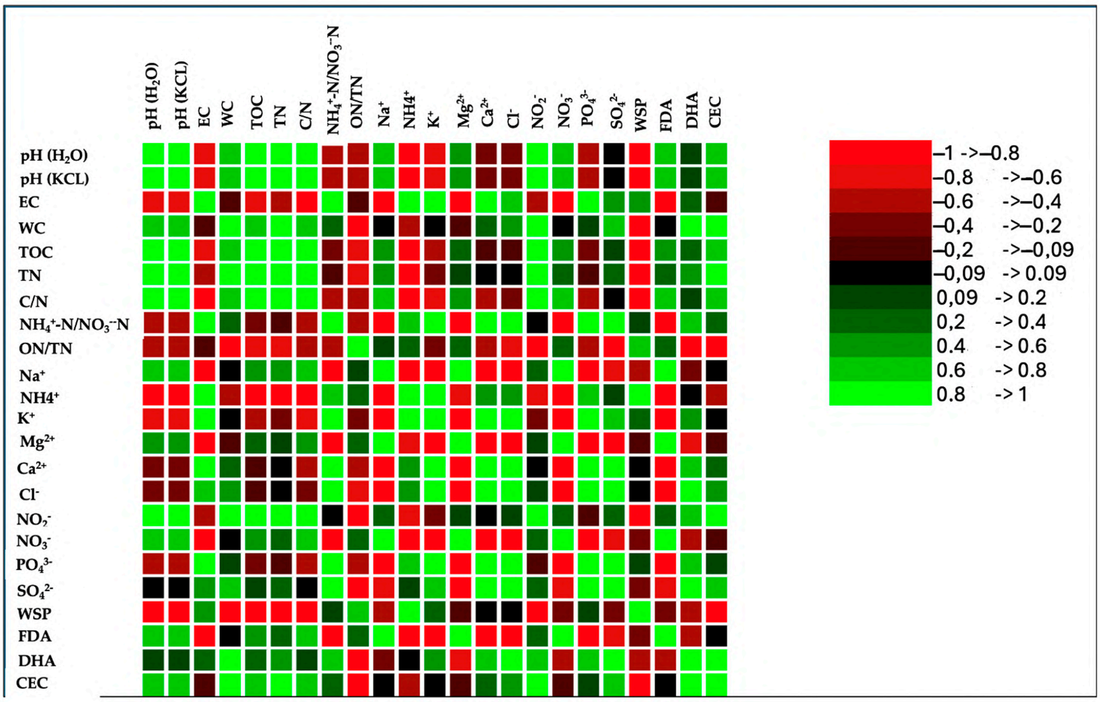


Figure 3. Correlation matrix (Pearson(n)) of chemical and biological properties and anions and cations at the end of the composting process (120 days). Green color and its shades, in the correlation matrix, indicate a positive correlation, signifying that the variables move in the same direction. On 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).

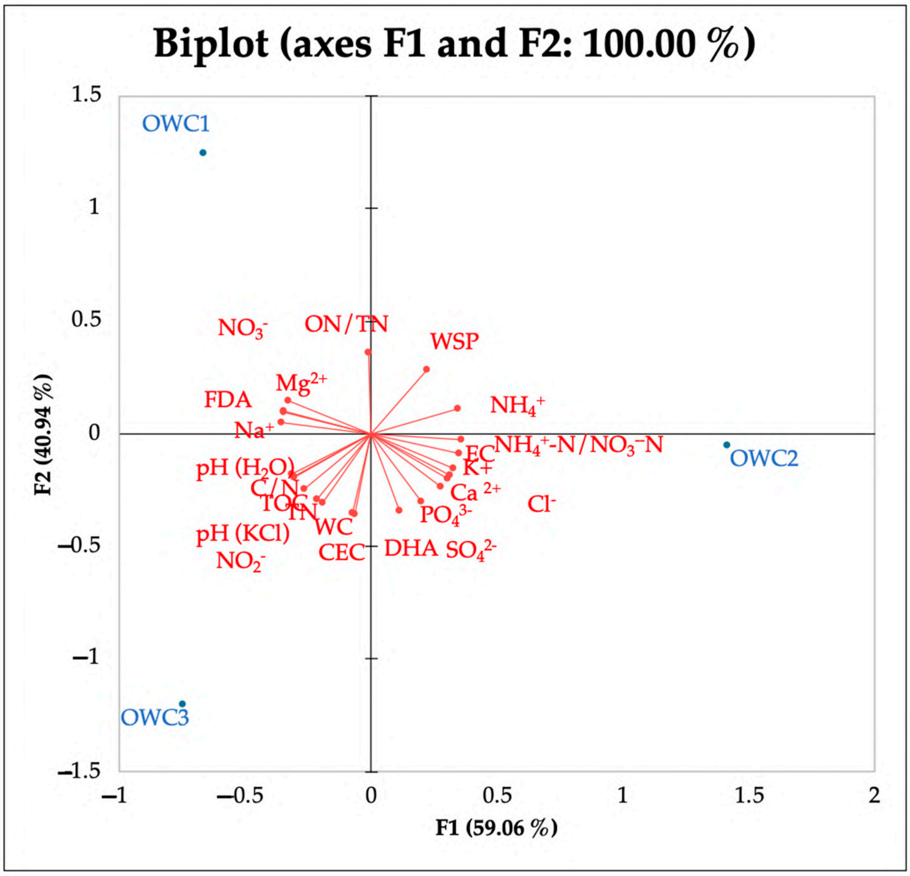


Figure 4. PCA of chemical and biological properties and anions and cations detected at the end of the composting process (120 days).

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	1.3×10^{5} ^{c} ± 0.21	4.4×10^{4} ^{c} ± 0.53	6.1×10^{4} ^{c} ± 0.47
S+OWC1	550 ^{b} ± 4.5	3.5 ^{b} ± 0.02	24.6 ^{b} ± 0.02	1.4×10^{5} ^{c} ± 0.19	1.2×10^{5} ^{b} ± 1.28	1.5×10^{5} ^{b} ± 0.22
S+OWC2	466 ^{c} ± 4.9	3.1 ^{c} ± 0.01	20.20 ^{c} ± 0.06	2.0×10^{5} ^{b} ± 0.13	1.1×10^{5} ^{b} ± 0.89	1.2×10^{5} ^{b} ± 0.27
S+OWC3	580 ^{a} ± 8.3	4.1 ^{a} ± 0.05	28.10 ^{a} ± 0.32	2.4×10^{5} ^{a} ± 0.11	1.5×10^{5} ^{a} ± 0.53	2.0×10^{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.

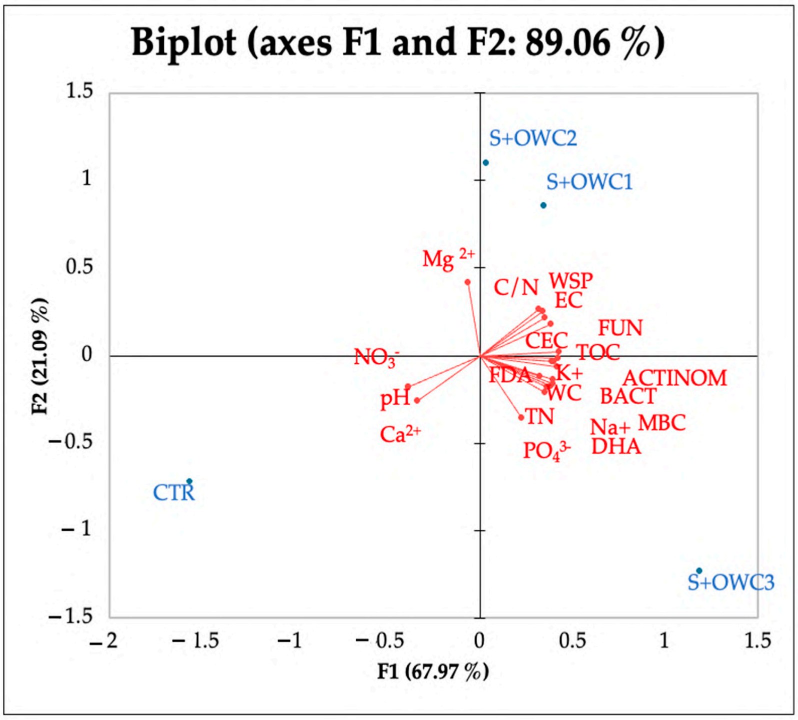


Figure 5. PCA of chemical and biological properties and anions and cations detected in soil amended with compost 1 (S+OWC1), compost 2 (S+OWC2), compost 3 (S+OWC3), and unamended soil (control, CTR). pH, electric conductivity (EC, dS m^{-1}), water content (WC, %), total organic carbon (TOC, %), total 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.).

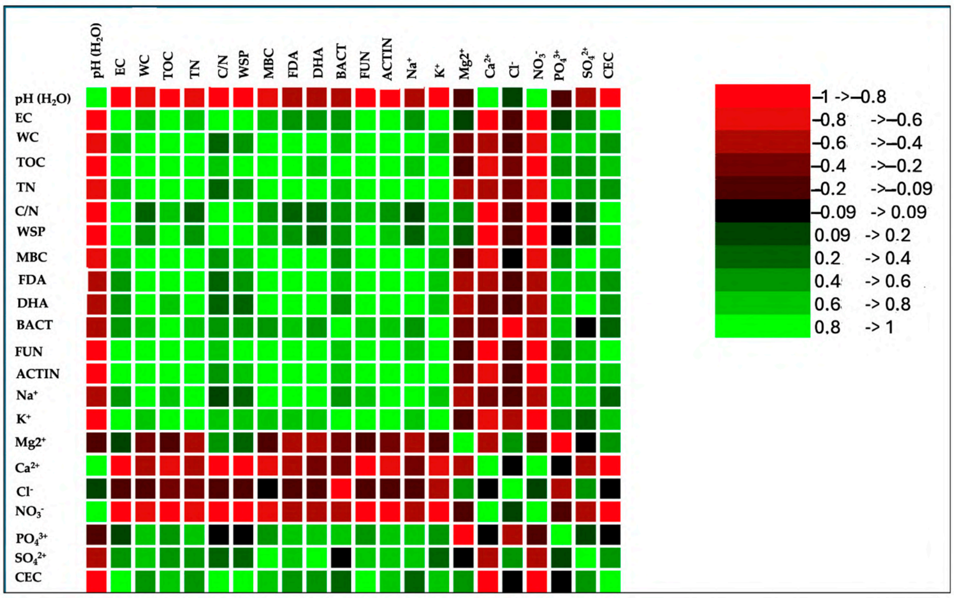
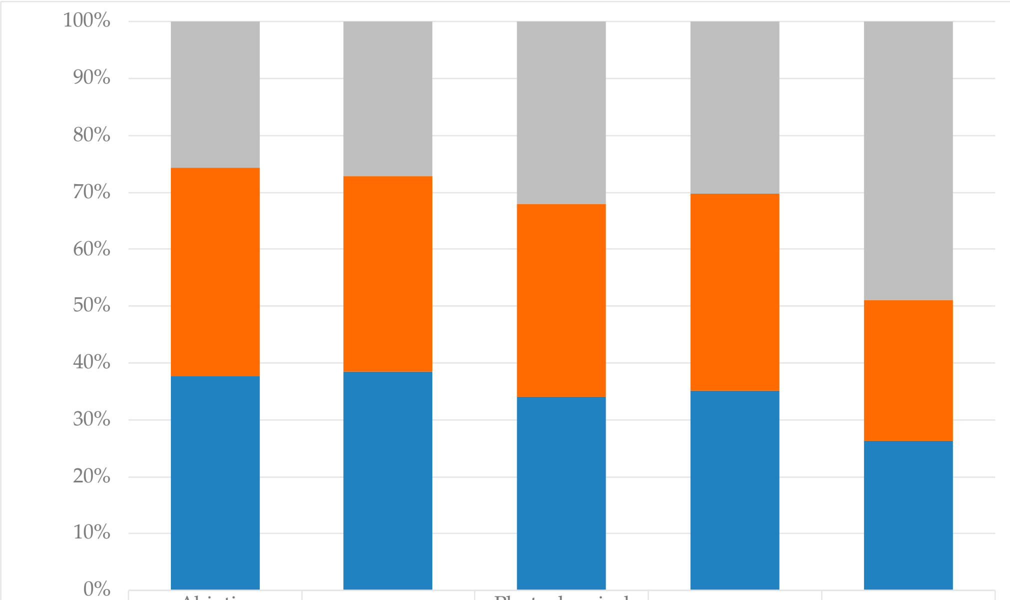


Figure 6. Correlation matrix of chemical and biological properties of soil plus compost 1 (S+OWC1), compost 2 (S+OWC2), compost 3 (S+OWC3), and unamended soil (control, CTR). Values in bold are different from 0 with a significance level alpha = 0.05. Green color and its shades in the correlation matrix 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.

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Chapter 7 - Transforming Agricultural and Sulfur Wastes into fertilizers: Assessing Short-Term Effects on Microbial Biodiversity via a Metagenomics Approach

Angela Maffia, Riccardo Scotti, Thomas Wood, Adele Muscolo, Alessandra Lepore, Elisabetta Acocella, Giuseppe Celano.

[1] Department of AGRARIA, 'Mediterranea' University of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria
angela.maffia@unirc.it; amuscolo@unirc.it

[2] NIAB, Cambridge Pathology, 93 Lawrence Weaver Road, Cambridge, United Kingdom
tom.wood@niab.com

[3] CREA Research Centre for Vegetable and Ornamental Crops, Via Cavalleggeri 51, 84098 Pontecagnano Faiano, Italy, riccardo.scotti@crea.gov.it

[4] Department od FARMACIA, University of Salerno, Via Giovanni Paolo II, Fisciano (SA) 84084. gcelano@unisa.it; alepore@unisa.t; e.acocella@studenti.unisa.it

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Article

Transforming Agricultural and Sulfur Waste into Fertilizer: Assessing the Short-Term Effects on Microbial Biodiversity via a Metagenomic Approach

Angela Maffia [1,†], Riccardo Scotti [2,3,†], Thomas Wood [2], Adele Muscolo [1,*], Alessandra Lepore [4], Elisabetta Acocella [4] and Giuseppe Celano [4]

1 Department of AGRARIA, 'Mediterranea' University of Reggio Calabria, Feo di Vito, 89122 Reggio Calabria, Italy; angela.maffia@unirc.it
2 NIAB, Cambridge Pathology, 93 Lawrence Weaver Road, Cambridge CB3 0LE, UK; riccardo.scotti@crea.gov.it (R.S.); tom.wood@niab.com (T.W.)
3 Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA), Research Centre for Vegetable and Ornamental Crops, Via Cavalleggeri 51, 84098 Pontecagnano Faiano, Italy
4 Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 84084 Fisciano, Italy; alepore@unisa.it (A.L.); e.acocella@studenti.unisa.it (E.A.); gcelano@unisa.it (G.C.)
* Correspondence: amuscolo@unirc.it
† These authors contributed equally to this work.

Abstract: Fungi and soil bacteria are vital for organic matter decomposition and biogeochemical cycles, but excessive synthetic fertilizer use contributes to soil degradation and loss of biodiversity. Despite this, about 97% of soil microorganisms are unculturable, making them difficult to study. Metagenomics offers a solution, enabling the direct extraction of DNA from soil to uncover microbial diversity and functions. This study utilized metagenomics to analyze the rhizosphere of two-year-old *Tonda di Giffoni* hazelnut saplings treated with synthetic NPK, composted olive pomace, and an innovative fertilizer derived from sulfur-based agro-industrial waste stabilized with bentonite clay. Using 16S rDNA for bacteria and ITS2 for fungi, Illumina sequencing provided insights into microbial responses to different fertilizer treatments. The results highlighted a significant increase in the abundance of beneficial microorganisms such as Thiobacillus, Pseudoxanthomonas, and Thermomyces, especially when organic materials were included. Additionally, microbial biodiversity improved with organic inputs, as shown by increased species richness (Chao1) and diversity (Bray-Curtis) greater than 20% compared with NPK and unfertilized soils (CTR). These findings emphasize the importance of organic fertilization in enhancing soil microbial health, offering a sustainable approach to improving soil quality and hazelnut productivity.

Keywords: organic waste; sulfur; olive pomace; metagenomics; biodiversity; microbiome; *Corylus avellana*

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1. Introduction

Hazelnut (*Corylus avellana* L.), a member of the Betulaceae family, ranks as the second most widely grown nut in the world, following only almonds [1]. The main hub of hazelnut production is located along the Black Sea coast of Turkey, Romania, Moldova, and Bulgaria, with other significant cultivation in Italy, the United States, and Spain [2]. To meet the growing consumer demand for hazelnuts and increase productivity, it is imperative to adopt strategies that ensure robust yields while preserving soil and the environment. Several studies have been conducted to improve the productivity and fertility of hazelnut cultivation [3–5] but as reported by Vincze et al. [6], a better understanding of the role of microbes in agroecosystem functioning in the context of plant growth and soil fertility is critical for sustainable agricultural production. Plant nutrition is closely influenced by the microbial community in the rhizosphere [7]. The soil rhizosphere is a micro-ecosystem in which the composition and structure of microorganisms can influence nutrient transformations in the soil, nutrient uptake by plants, and, thus, plant growth and development [8]. According to Roeland et al. [9], the rhizosphere functions as a unique region where the interaction between the plant, root, and soil microbiome takes place; in fact, as stated by Philippot et al. [10], the rhizosphere is one of the most dynamic interfaces on Earth.

In recent years, in response to climate change and the widespread use of synthetic/mineral fertilizers, the effects of different fertilization techniques on the rhizosphere microbial community have begun to be studied [7,11,12].

A study by Chavez-Romero et al. [13] states that the application of organic fertilizers has beneficial effects compared to inorganic fertilizers in promoting the diversity of soil microbial communities and enriching the soil with organic carbon, nitrogen, and other nutrients. Dai X.B et al. [14] observed that the increased application of synthetic nitrogen and phosphate fertilizers changed the composition of the soil microbial community by reducing the abundance of gram-positive bacteria such as actinomycetes. Guo et al. [15] found that synthetic fertilization negatively influenced microbial communities in the rhizosphere, and that positive effects on soil microbial communities were observed with the addition of organic fertilizer compared to chemical fertilization [16]. Similarly, Legrand et al. [17] found greater bacterial richness and uniformity with manure application compared to chemical fertilization. In contrast, Orr et al. [18] observed no discernible influence on the microbial community when comparing conventional and organic farming systems. The composition and biodiversity of the observed microbiota were attributed exclusively to environmental and soil chemical variables.

Although several previous studies have focused mainly on long-term field experiments and organic fertilizers can have an impact even in the short term, within a single growing season, especially on crop productivity [19,20]. Nevertheless, no study has yet been conducted on the evaluation of mineral and organic synthetic fertilizers on hazelnut saplings in the short term, specifically on the influence of these fertilizations on rhizosphere microbial processes. Studies that have employed culture-based methods [21,22] capture only a limited fraction of the overall microbial diversity. In contrast, next-generation sequencing (NGS) techniques offer a powerful approach to achieving a more comprehensive understanding of microbial community composition and diversity [23].

The aim of this study is to evaluate the short-term changes in microbial diversity and community in the rhizosphere of hazelnut saplings. The rhizosphere microbial community of unfertilized hazelnut saplings was compared with the rhizosphere microbial community of hazelnut saplings fertilized with synthetic commercial NPK fertilizer (NPK), composted olive pomace (OP), sulfur bentonite (SB), and sulfur bentonite + composted olive pomace (SBOP).

SB and SBOP are new fertilizers obtained from agro-industrial wastes composed as follows: SB comprises elemental sulfur (S) residue from the hydrocarbon refining process stabilized with bentonite clay, without composted olive pomace; SBOP comprises elemental S residue from the hydrocarbon refining process stabilized with bentonite clay, with the addition of composted olive pomace. The effectiveness of sulfur-based fertilizers, both with and without an organic component, has been demonstrated in different studies [24,25], but no studies up to now have been carried out on tree species. This study aims to verify if fertilization yields different outcomes compared to the previously studied horticultural plants. The novelty of this study lies in its integration of advanced metagenomic techniques with the evaluation of innovative organic fertilizers to assess their impact on soil microbial communities. By employing 16S rDNA and ITS2 sequencing to profile bacterial and fungal communities, the study provides a detailed view of microbial diversity and functions in the rhizosphere, especially in response to different fertilizers. The application of advanced metagenomic techniques to investigate the effects of different fertilizations on the soil microbiome addresses a critical challenge in soil microbiology: the inability to culture approximately 97% of bacterial species due to limited knowledge of their specific cultivation requirements, including nutritional needs, the physicochemical conditions of their natural environment, and the complex symbiotic or parasitic relationships within microbial communities [26]. The combination of innovative fertilizer evaluation, cutting-edge metagenomic analysis, and a focus on sustainable practices provides a fresh perspective on managing soil health in hazelnut agriculture and contributes to broader efforts in sustainable farming practices.

2. Materials and Methods

2.1. Fertilizers Manufacturing

The olive pomace compost used in this study was produced at the farm composting plant of the Nuovo Cilento agricultural company, in San Mauro Cilento (SA), Italy.

The chemical characteristics of olive pomace compost are reported in Table 1 and analyzed according to Muscolo et al. [24]. The compost highlights an alkaline pH, moderate electrical conductivity, good organic matter content, and the presence of essential nutrients (anions and cations).

Table 1. Chemical properties of composted olive pomace. The data are the mean of three replicates ± standard deviation.

Chemical Properties	Value
pH (H_2O)	7.8 ± 0.04
EC (mS cm^{-1})	2.6 ± 0.27
Moisture (g kg^{-1} fw)	383 ± 0.3
C% (g kg^{-1} dw)	426 ± 0.4
Total N (g kg^{-1} dw)	19.6 ± 0.1
C/N	21.7 ± 0.3
Na^+ (mg g^{-1} dw)	4.35 ± 0.02
$NH4^+$ (mg g^{-1} dw)	1.50 ± 0.01
K^+ (mg g^{-1} dw)	9.13 ± 0.06
Mg^{2+} (mg g^{-1} dw)	1.45 ± 0.43
Ca^{2+} (mg g^{-1} dw)	14.06 ± 1.4
Cl^- (mg g^{-1} dw)	0.02 ± 0.5
$PO4^{3-}$ (mg g^{-1} dw)	0.40 ± 0.4
$SO4^{2-}$ (mg g^{-1} dw)	0.18 ± 2.9
WSB (mg TAE g^{-1} dw)	2.5 ± 0.05

The synthetic fertilizers were produced by the Steel Belt System s.r.l and the process of manufacturing is reported in Panuccio et al. [27]. The composition of different fertilizers is reported in Table 2.

Table 2. Composition of different fertilizers.

Fertilizers	Composition
Sulfur Bentonite + Olive Pomace (SBOP)	5% of composted olive pomace recovered using a two-phase oil mill 10% of bentonite clay 85% of elemental sulfur.
Sulfur Bentonite (SB):	90% of elemental sulfur 10% of bentonite clay.

Table 2. *Cont.*

Fertilizers	Composition
Composted Olive Pomace (OP)	34% of composted olive pomace recovered using a two-phase oil mill 33% of buffalo manure 33% of a mixture consisting of wood defibrate and olive leaves.
Synthetic fertilizer (NPK)	20% of N 10% of P_2O_5 10% of K_2O

2.2. Experimental Design

In February 2023, two-year-old hazelnut saplings var. *Tonda di Giffoni* were placed in 30-L capacity pots filled with sandy loam soil (64% sand, 28% loam, and 8% clay) according to the Agricultural Organization of the United Nations (FAO) [28]. The soil contained an organic matter content of 20 g $\times$ kg^{-1}, a total nitrogen concentration of 1 g $\times$ kg^{-1}, and a carbon-to-nitrogen ratio of 12.

Four replications were made per treatment for a total of 20 pots, with the following doses of each treatment per pot:

- 45 g of composted olive pomace (OP).
- 5 g of sulfur bentonite + olive pomace (SBOP).
- 5 g of sulfur bentonite (SB).
- 4.2 g of synthetic fertilizer (NPK).
- Unfertilized soil (CTR).

For each treatment, fertilizer was applied twice before flowering in February and before vegetative growth in June. To limit weed development and avoid excessive soil overheating, the pots were isolated by placing them in larger-diameter containers (50 cm), and the surface soil and defined chamber were filled with expanded clay (Figure 1). The soil water content, through irrigation with potable water, was always maintained at 70% of field capacity.

Figure 1. Experimental site and pots with *Corylus avellana* plants and different treatments.

2.3. Sample Collection and DNA Extraction

In July, rhizosphere sampling was performed using the protocol of Simmon et al. [29], with some modifications: 2 mm of soil attached to the roots was left and a representative section was cut and placed in a 50 mL tube with 25 mL of phosphoric buffer, centrifuged for 10 min at 4000× *g* at 4 °C, and the root was removed to obtain the rhizosphere fraction. Rhizosphere DNA was extracted using a commercial kit DNeasy PowerSoil Pro kit, (Qiagen, Hilden, Germany), following the protocol with one modification: 10 mg of skim milk was added to all the samples, as suggested by Hoshino et al. [30], to increase the DNA extraction yield from volcanic soil. The final DNA concentrations were determined using a NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, NC, USA), and the DNA quality was checked using 1% agarose gel electrophoresis on a Luminescence Image Analyzer System (LAS 4000; ImageQuant, GE Healthcare Life Sciences, Little Chalfont, UK) (Figure 2).

Figure 2. DNA quality control of DNA extraction samples obtained using a Luminescence Image Analyzer System (LAS).

DNA samples were screened for the V3–V4 hypervariable regions via PCR using primers 341F (5′-GCGGTAATTCCAGCTCCAA-3′) and 806R (5′-GGACTACNNGGGTATCTAAT) for 16S rDNA. For ITS2, the primers ITS3-2024F (5′-GCATCGATGAAGAACGCAGC-3′) and ITS4-2409R (5′-TCCTCCGCTTATTGATATGC-3′) were used [31]. All PCR reactions were carried out with 15 µL of Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA); 2 µM of forward and reverse primers, and about 10 ng of template DNA. Thermal cycling consisted of initial denaturation at 98 °C for 1 min, followed by 30 cycles of denaturation at 98 °C for 10 s, annealing at 50 °C for 30 s, and elongation at 72 °C for 30 s. Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Lastly, the library was sequenced on an Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA) and 250 bp paired-end reads were generated. The DNA library preparation and sequencing were performed by Novogene Co, Ltd. (Beijing, China, http://www.novogene.com/ accessed on 1 May 2024). The raw sequence files generated (fastq files) underwent quality control analysis with FastQC.

2.4. Data Analysis

The NGS datasets were analyzed using the EBI Metagenomics service pipeline, which provides quality control, taxonomic analysis based on SSU rDNA sequences, and sequence assembly (MGnify. 2023. "Analysis Pipeline V5.0" 9 November 2023. https://docs.mgnify.org/src/docs/analysis.html accessed on 9 November 2023).

2.5. Biodiversity Assessment and Statistical Analysis

Sequence data from the rhizosphere soil community was analyzed using Microbiome Analyst 2.0 (https://www.microbiomeanalyst.ca/) [32]. Microbial biodiversity was assessed to quantify differences between groups at two levels: within-samples (alpha-diversity) and between samples (beta-diversity) [33]. From the different six measures supported in Microbiome Analyst described by Chong et al. [34], the Chao1 index [35] and Shannon index [36,37] were selected. From the five different beta-diversity indexes supported by Microbiome Analyst, "Bray–Curtis dissimilarity" [38] was utilized. The index measures the compositional dissimilarly between the microbial communities based on the counts of each sample. Microbiome Analyst can measure Beta-diversity using PCoA or nonmetric multidimensional scaling (NMDS); for this study, PCoA was selected because it maximizes the linear correlation between samples [34]. For identifying microbial taxa that were significantly different between groups, LEfSe (Linear discriminant analysis Effect Size), a non-parametric statistical method, was selected. A significance level of $p < 0.05$ and LogLDA score of ± 2 was applied. The jveen tool (INRAE, Toulouse, France) [39] was used to compare the genera of fungi and bacteria that were found to be statistically significant using LeFSE. 'Class' level was selected for bacteria and fungi for generating the Heatmap outputs. The distance between data points in the clustering input is measured with the standard Euclidean (as-the-crow-files) distances. All sequences were deposited at the European Nucleotide Archive (ENA, http://www.ebi.ac.uk/ena accessed on 15 January 2024) under project number PRJEB70816 for bacteria and PRJEB68325 for fungi.

3. Results

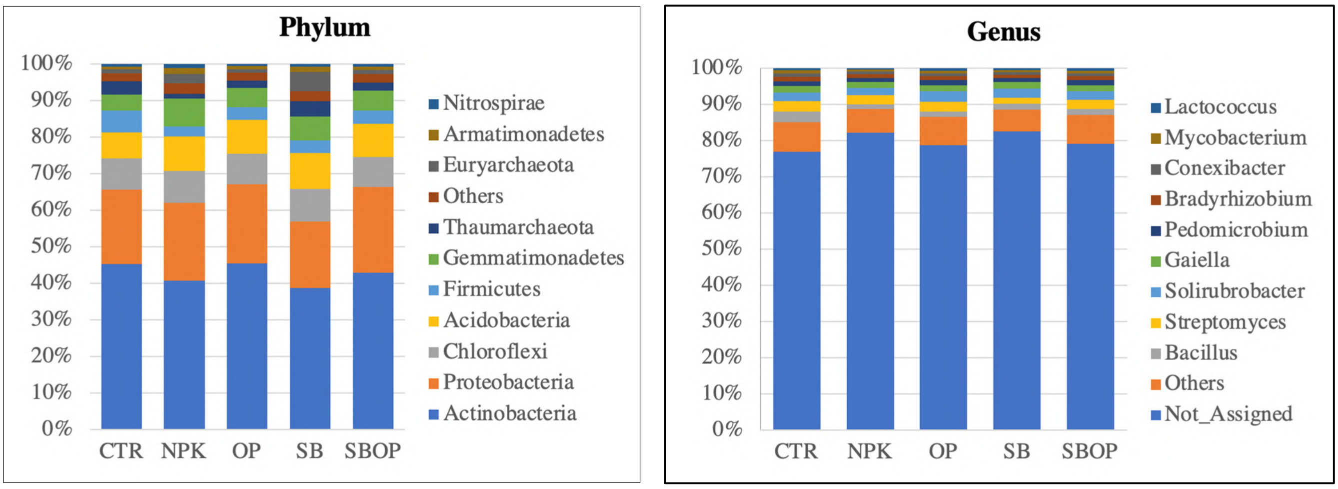
Sequencing generated a total of 1656509 (16S rDNA) and 1749133 (ITS2) sequences, respectively, for 20 samples, classified into 1202 OTUs for the 16S rDNA and 1642 OTUs for the ITS2.

3.1. Bacteria Taxa Abundance

Results showed that amongst the 10 most abundant bacterial phyla, *Actinobacteria* were the most dominant, constituting 46% in rhizosphere soil treated with OP, 45% in control soil, 43% in SBOP-treated soil, 41% in soil with NPK, and 39% in SB-treated soil (Figure 3a).

The second most abundant phylum was the *Proteobacteria*: representing 23% in SBOP-treated soil, 22% in soil with OP, 21% with NPK, 20% in CTR soil, and 18% in SB-treated soil. *Acidobacteria* increased in all treated soils, compared to CTR (7%), especially with SB (10%).

About 76% of the genus classification was not identified. The genus *Bacillus* was most prevalent in CTR control plants (3%) but decreased with SB (2%) and SBOP (2%) and accounted for only 1% in NPK and OP treatments. Contrastingly, the genus *Streptomyces* was present at an equal percentage of 3% in all treatments except for SB-treated plants where it decreased to 2%. (Figure 3b).

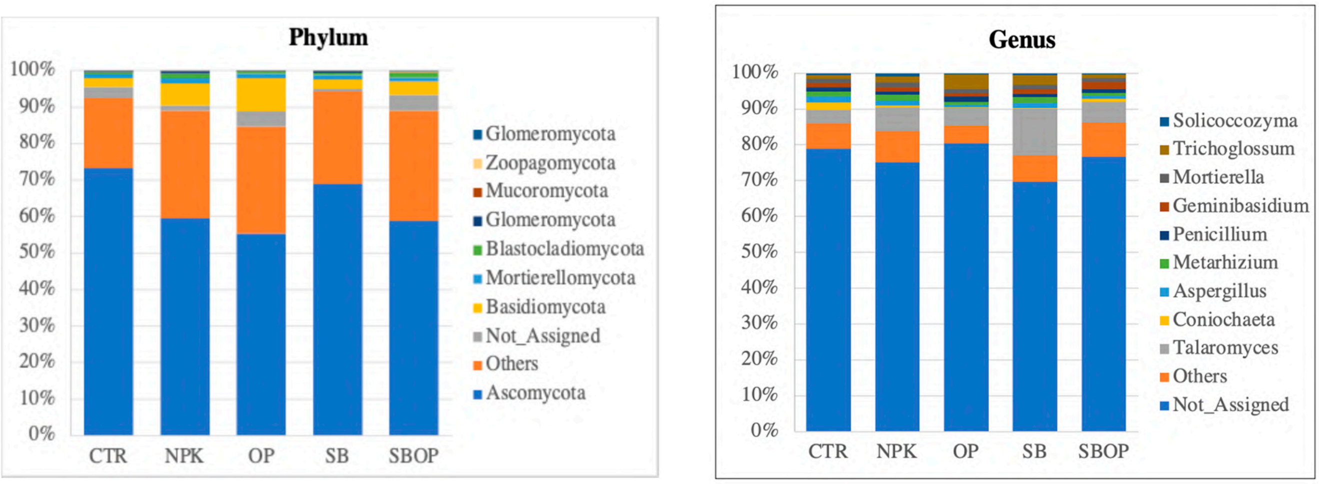


(**a**) (**b**)

Figure 3. The top 10 bacteria taxa relative abundance for phylum (**a**) and genus (**b**) of soils treated with synthetic fertilizer (NPK), olive pomace (OP), sulfur bentonite (SB), and sulfur bentonite + olive pomace (SBOP). CTR is unfertilized soil.

3.2. Fungi Taxa Abundance

Taxonomic classification showed that the most abundant phylum among the samples was *Ascomycota*. The unfertilized CTR soils had the highest percentage of *Ascomycota* fungi (73%), followed by SB treatment (69%), SBOP (59%), NPK (59%), and OP (55%). In addition, an abundance of organisms classified as others, because they do not belong to the kingdom of fungi, was detected, mainly belonging to the phylum *Streptophyta*. Fungi belonging to the phylum *Streptophyta* showed an increase compared to the control (19%) across all treatments: SB (25%), NPK (29%), OP (29%), and SBOP (30%). Fungi from the phylum *Basidiomycota* were predominantly found in the OP treatment (9%) (Figure 4a).



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

Figure 4. The top 10 fungi taxa abundance for phylum (**a**) and genus (**b**).

About 76% of the genus classification was not identified. The most abundant genus for all samples was *Talaromycetes*, especially in those treated with SB (13%). This relative abundance decreased in soils fertilized with NPK (7%), SBOP (6%), OP (5%) and in control soil (4%) (Figure 4b).

3.3. Bacteria Alpha and Beta Diversity

Species richness calculated using the Chao1 index for bacteria OTUs showed that all treatments resulted in an increase compared to control plants (without fertilization) (Figure 5a). Specifically, the largest increase was observed in plants treated with sulfur bentonite + olive pomace fertilizer (SBOP), followed by those treated with olive pomace (OP). Treatment with the synthetic fertilizer (NPK) also resulted in a statistically significant increase, while a smaller increase was noted for sulfur bentonite (SB) treatment (Figure 5a). The results of the post-hoc pairwise comparison (multiple group only) revealed statistically significant differences ($p < 0.05$) between several treatments. Specifically, there was a significant difference between OP and NPK ($p = 0.034$), OP and SB ($p = 0.039$), and CTR and OP ($p = 0.039$). The differences in community composition between samples calculated with the Bray–Curtis index showed a dissimilarity of 35.6% for axis one and a dissimilarity of 16.2% for axis two. (Figure 5c). Although no clear clustering pattern was shown using unsupervised PCoA on Bray–Curtis dissimilarity, the principal coordinate two highlights a trend along the sample distribution, mainly driven by the use of compost on one side and mineral fertilization and sulfur use on the other side. From the result of pairwise PERMANOVA analysis and the multi-testing adjustment, based on the Benjamini–Hochberg procedure (FDR), a statistically significant difference for a p value < 0.05 can be seen between the CTR vs. NPK treatments with a p value of 0.037, and OP vs. NPK with a p value of 0.046 (Supplementary Material Table S1).

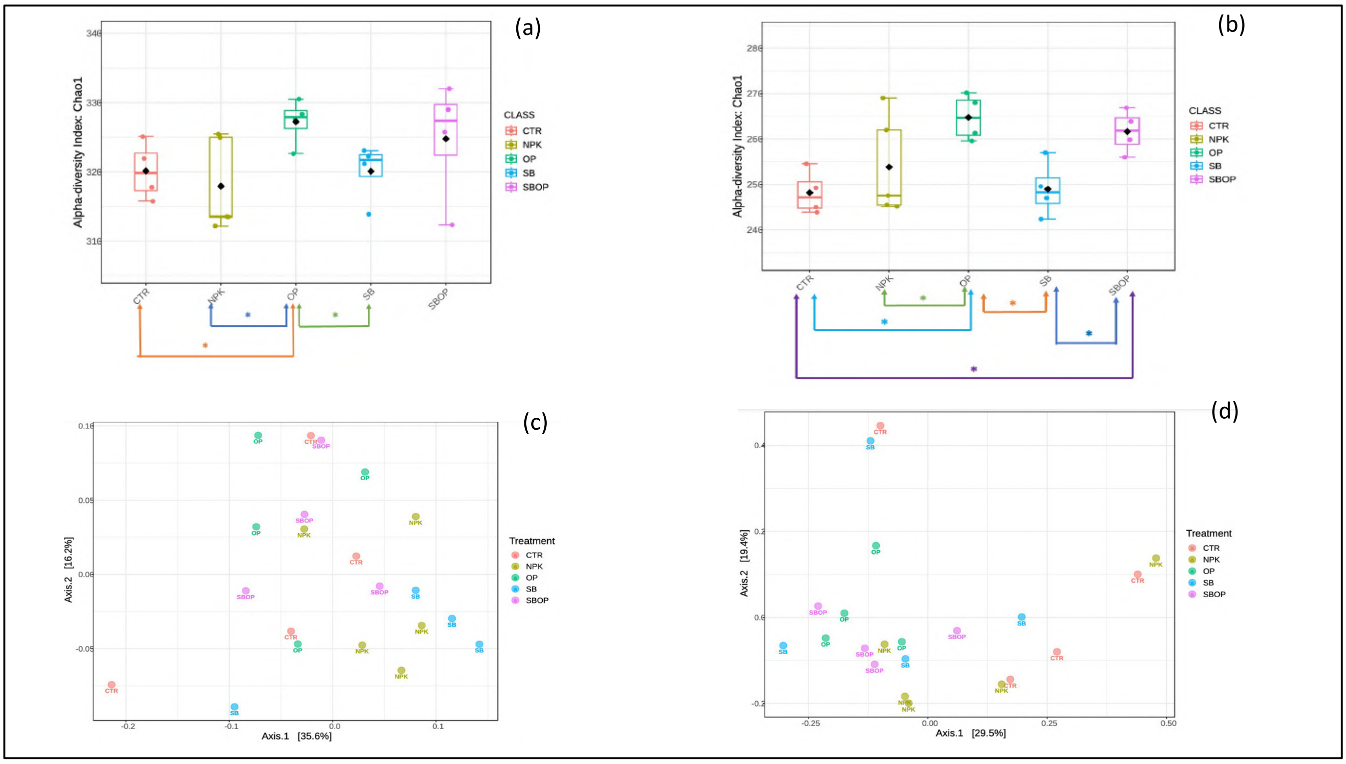


Figure 5. Chao1 index of bacteria (**a**) and fungi (**b**). Statistically significant differences are expressed with (*) for a p value < 0.05 through post-hoc pairwise comparison (multiple groups only). Principal Coordinate analysis (PCoA) of bacteria (**c**) and fungi (**d**) calculated with the Bray–Curtis dissimilarity of soils treated with synthetic fertilizer (NPK), olive pomace (OP), sulfur bentonite (SB), and sulfur bentonite + olive pomace (SBOP). CTR is unfertilized soil.

3.4. Fungi Alpha and Beta Diversity

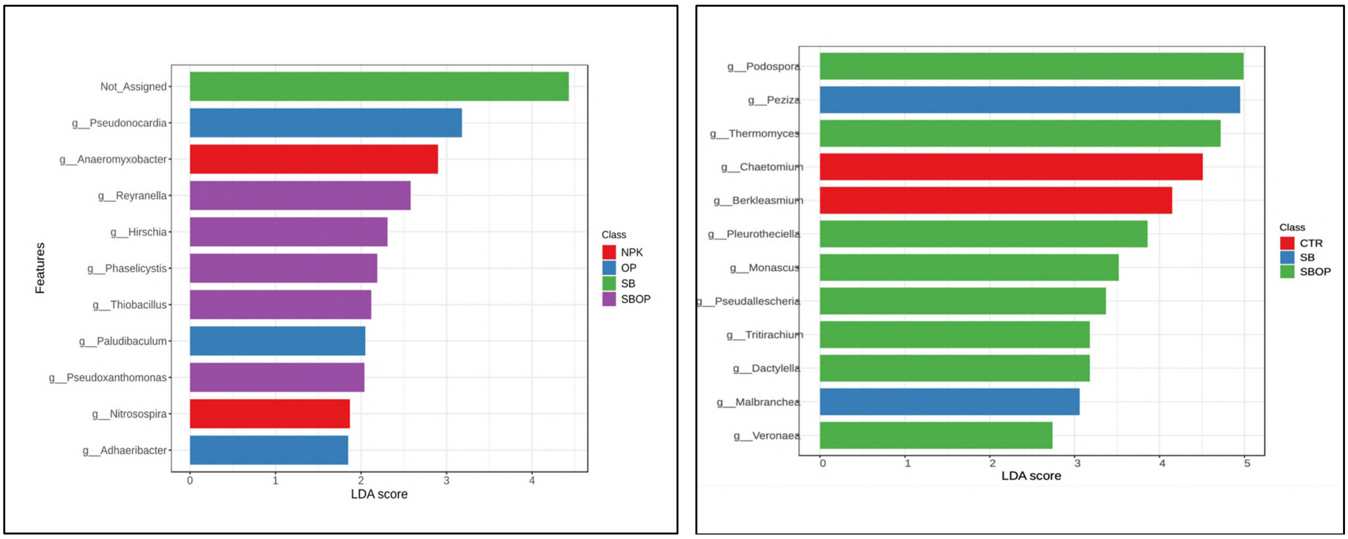
Species richness, calculated using the Chao1 index for fungal OTUs, demonstrated an increase in all treatments compared to control plants (without fertilization). Specifically, the olive pomace (OP) treatment exhibited the greatest increase in this index, followed by sulfur bentonite + olive pomace (SBOP), and then the synthetic fertilizer (NPK) (Figure 5b).

From the results of the post-hoc pairwise comparison (multiple group only), statistically significant differences ($p < 0.05$) were observed between several treatments. These included OP vs. CTR ($p = 0.0033$), SBOP vs. CTR ($p = 0.0073$), OP vs. SB ($p = 0.0079$), and SBOP vs. SB ($p = 0.018$).

The dissimilarity in community composition between samples calculated using the Bray–Curtis dissimilarly showed a dissimilarity of 29.5% for axis one and a dissimilaritly of 19.4 for axis two (Figure 5c). Although no clear clustering pattern is shown using unsupervised PCoA on the Bray–Curtis index, the second principal coordinate two highlights a trend along the sample distribution mainly driven by the use of compost on one side and mineral fertilization and sulfur use on the other side. From the result of pairwise PERMANOVA analysis and the multi-testing adjustment based on the Benjamini–Hochberg procedure (FDR), a statistically significant difference for a p value < 0.05 can be seen between treatments of OP vs. NPK with a p value of 0.032, and OP vs. CTR with a p value of 0.047 (Supplementary Materials Table S2).

3.5. Comparison Analysis with LEfSe, a Heatmap of Relative Abundance, and a Veen Diagram

For bacteria, the linear discriminant analysis effect size (LEfSe) to feature level with the p-value cut-off of 0.05 and LogLDA score of 2.0 identified a total of 44 significant features (Supplementary Materials Table S3). There were 10 significant genera observed (Figure 6a).



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

Figure 6. LEfSe results on soil bacterial (**a**) and fungal (**b**) communities. Histogram of the LDA scores computed for features differentially abundant on different treatments. LEfSe scores can be interpreted as the degree of consistent difference in relative abundance between features of analyzed bacterial and fungal communities. The histogram, thus, identifies which clades among all those detected as statistically and biologically differential explain the greatest differences between genus communities.

From the Venn diagram analysis, bacteria belonging to *Pseudonocardia*, *Phaselicystis*, *Hirschia*, *Paludibaculum*, *Nitrosospira*, *Anaeromyxobacter*, *Adhaeribacter*, and *Reyranella* were found to be common across all treatments and the unfertilized soil (CTR) (Figure 7). The genus *Thiobacillus* was common to treatments with OP, SB, and SBOP. In contrast, the genus *Pseudoxanthomonas* was found to be common between treatments with OP and SBOP, as well as in the unfertilized soil (CTR) (Figure 7).

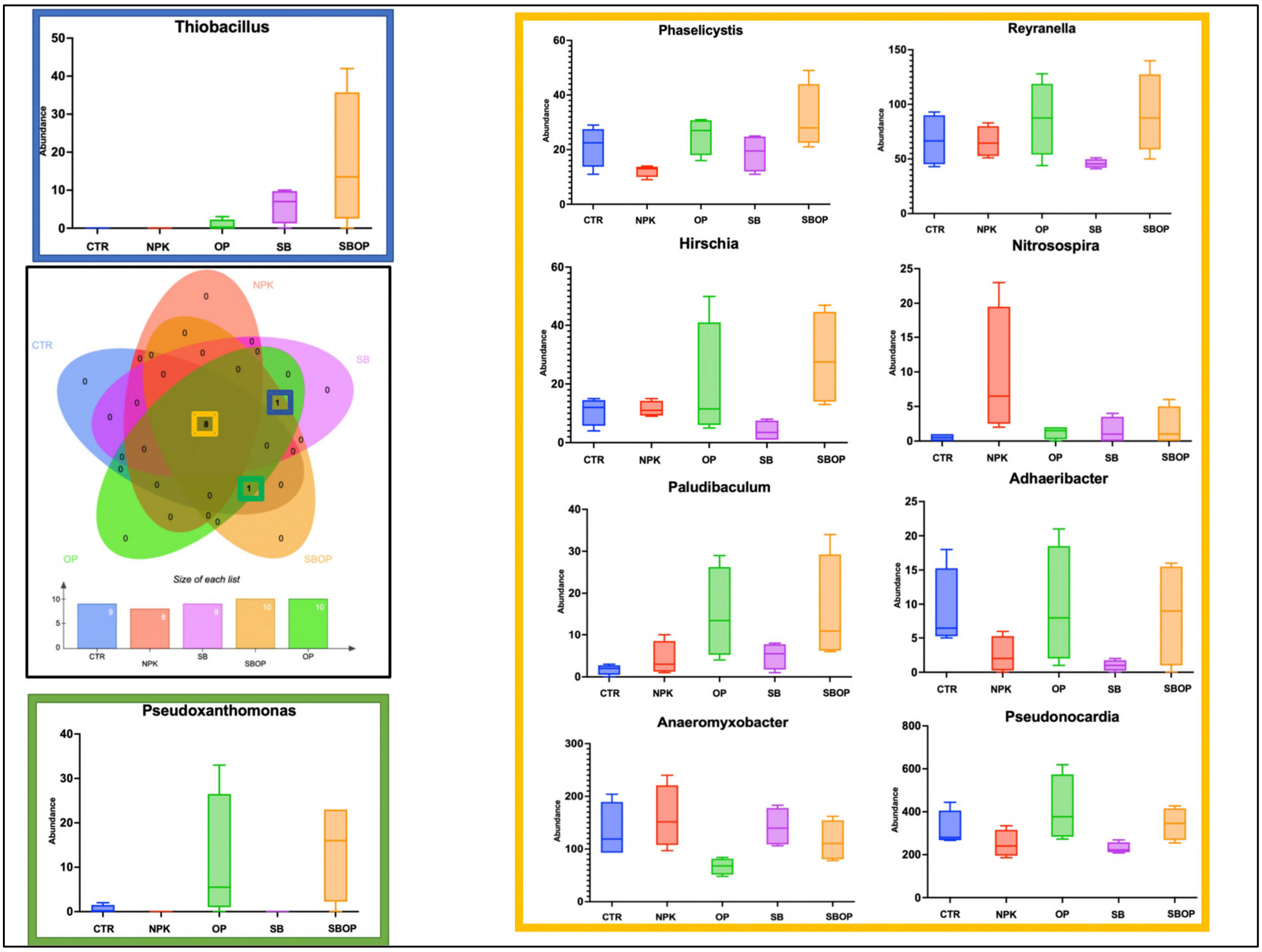


Figure 7. Veen diagram of the genus of bacteria which were found to be statistically significant from linear discriminant analysis effect size (LEfSE) and related box plots.

A histogram of the LDA scores was computed for features that were differentially abundant on different treatments. The LEfSe scores can be interpreted as the degree of consistent difference in relative abundance between features of analyzed bacterial and fungal communities (Figure 6). The histogram, thus, identifies which clades among all those detected as statistically and biologically differential explain the greatest differences between genus communities.

The genus *Pseudonocardia* appeared more abundant in the OP treatment, with no significant differences observed compared to other treatments. Meanwhile, the genus *Thiobacillus* was notably absent in both control soil and NPK-fertilized plants but showed an increase with all other treatments, particularly with SBOP. The *Hirschia*, *Paludibaculum*, and *Reyranella* genera increased in abundance when organic components were added to fertilizers, notably in SBOP and OP treatments. Conversely, the genus *Pseudoxanthomonas* showed an increase compared to the control in OP and SBOP treatments, while decreasing in plants treated with NPK and SB. *Nitrosospira* bacteria were more abundant in NPK-treated soil. However, bacteria belonging to the genus *Anaeromyxobacter* decreased in OP and SBOP treatments. *Adhaeribacter* bacteria decreased across all treatments, with a significant reduction observed in mineral treatments with SB and NPK (Figure 7).

For Fungi, the linear discriminant analysis effect size (LEfSe) was employed up to the taxonomy level feature level, with a p-value cutoff of 0.05 and a LogLDA score of 2.0, resulting in a total of 28 significant features (Supplementary Materials Table S4). Among these, 12 significant genera were identified (Figure 6b).

From the Venn diagram analysis (Figure 8), it was observed that the fungi genera *Podospora*, *Chaetomium*, and *Berkleasmium* are common across all treatments and the unfertilized soil (CTR). The genera *Thermomyces, Pseudoallescheria*, and *Peziza* were common across all soils treated with fertilizers. The genus *Malbranchea* was common to the unfertilized soil (CTR), OP, SB, and SBOP treatments but not in NPK treatment. On the other hand, the genus *Veronae* was common to the unfertilized soil (CTR) and all other treatments except SB. Soil treatments with NPK, OP, and SBOP shared the genera *Monascus* and *Pleuroteciella*. Only treatments with OP and SBOP shared the genera *Tritirachium* and *Dactylella*. Fungi of the genus *Pseudoallescheria*, *Thermomyces*, and *Podospora* were predominantly in soil with organic components, such as OP and SBOP fertilizers.

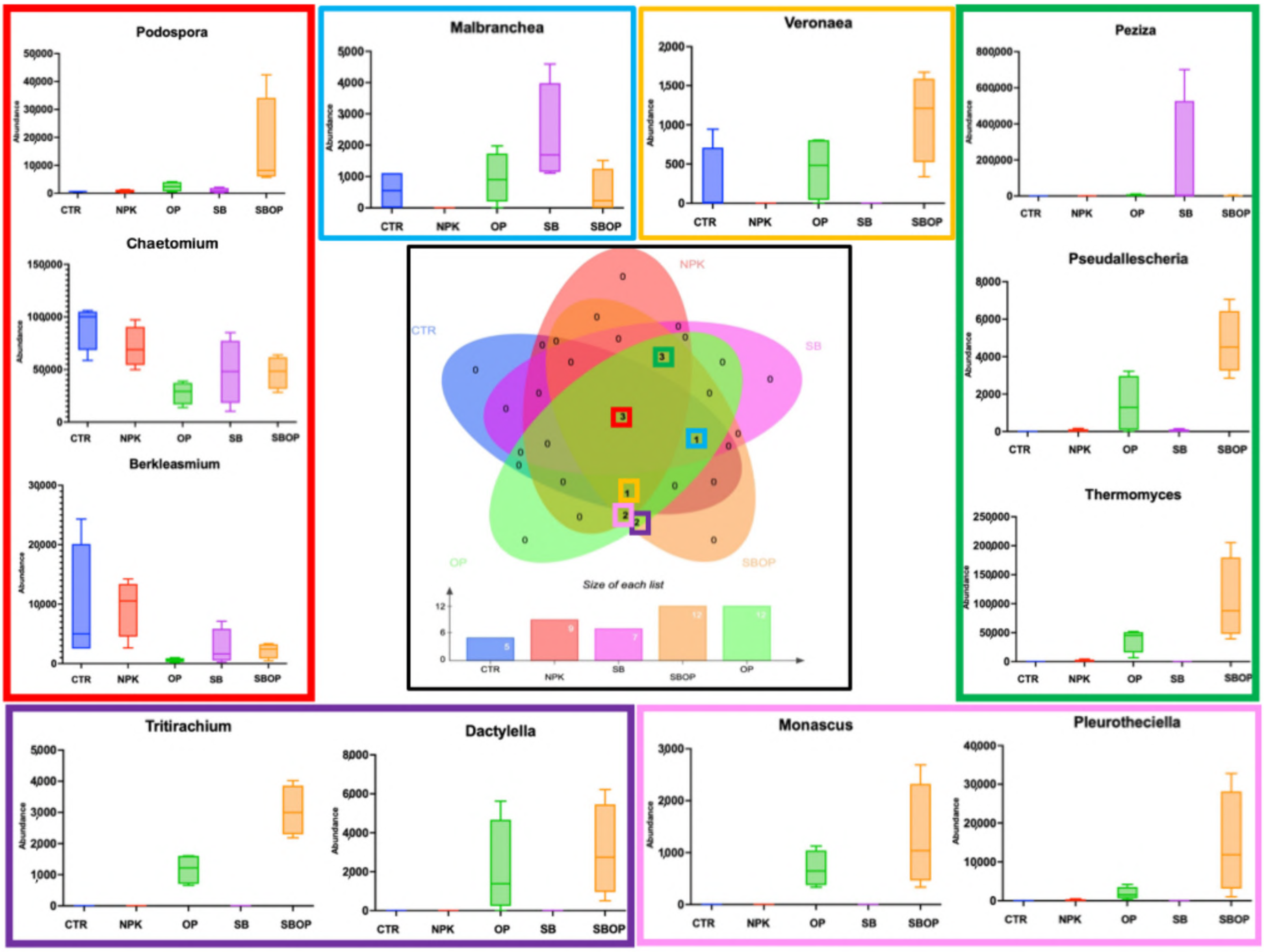


Figure 8. Veen diagram of the genus of fungi which were found to be statistically significant from linear discriminant analysis effect size (LEfSE) and related box plots.

Conversely, fungi of the genus *Chaetomium* and *Berkleasmium* behaved consistently across the OP, SB, and SBOP fertilizer inputs, decreasing compared to soil without fertilizer (CTR) and NPK synthetic fertilizer.

Meanwhile, fungi belonging to the genus *Malbranchea* and *Peziza* showed a significant increase in soil fertilized with SB compared to all other treatments. In contrast, fungi of the genus *Veronae* decreased in treatments with OP and SB compared to unfertilized soil (CTR) and NPK-fertilized soil but increased in soil fertilized with SBOP (Figure 8).

Heatmaps representing bacterial diversity at a class level (Figure 9) indicated that SBOP treatment showed high abundance for *Gammaproteobacteria* and *Bacilli*, suggesting that this treatment created a favorable environment for the proliferation of these classes. In contrast, the SB treatment showed a low abundance of *Alphaproteobacterial* and *Actinobacteria*. Treatment with SB varied between replicates, showing a less uniform response than the other treatments. OP treatment appears less favorable for the proliferation of *Acidobacteria* and *Anaerolineae*, indicating a possible negative impact of OP treatment on this class of bacteria. The NPK treatment showed a variable abundance, especially in the classes *Betaproteobacteria* and *Clostridia*. Soils without treatment (CTR) showed that the abundance of the bacterial classes is generally moderate to low, especially for the classes *Planctomycetacia* and *Verrucomicrobiae*. The dendrogram shows how the bacterial classes are grouped according to their abundance. Classes such as *Gemmaproteobacteria* and *Bacilli* tend to cluster together, indicating a similar response to treatments. In contrast, the upper dendrogram shows how samples with similar abundance patterns cluster together. This clustering confirms that SBOP-treated samples tend to have more similar abundance profiles than other treatments.

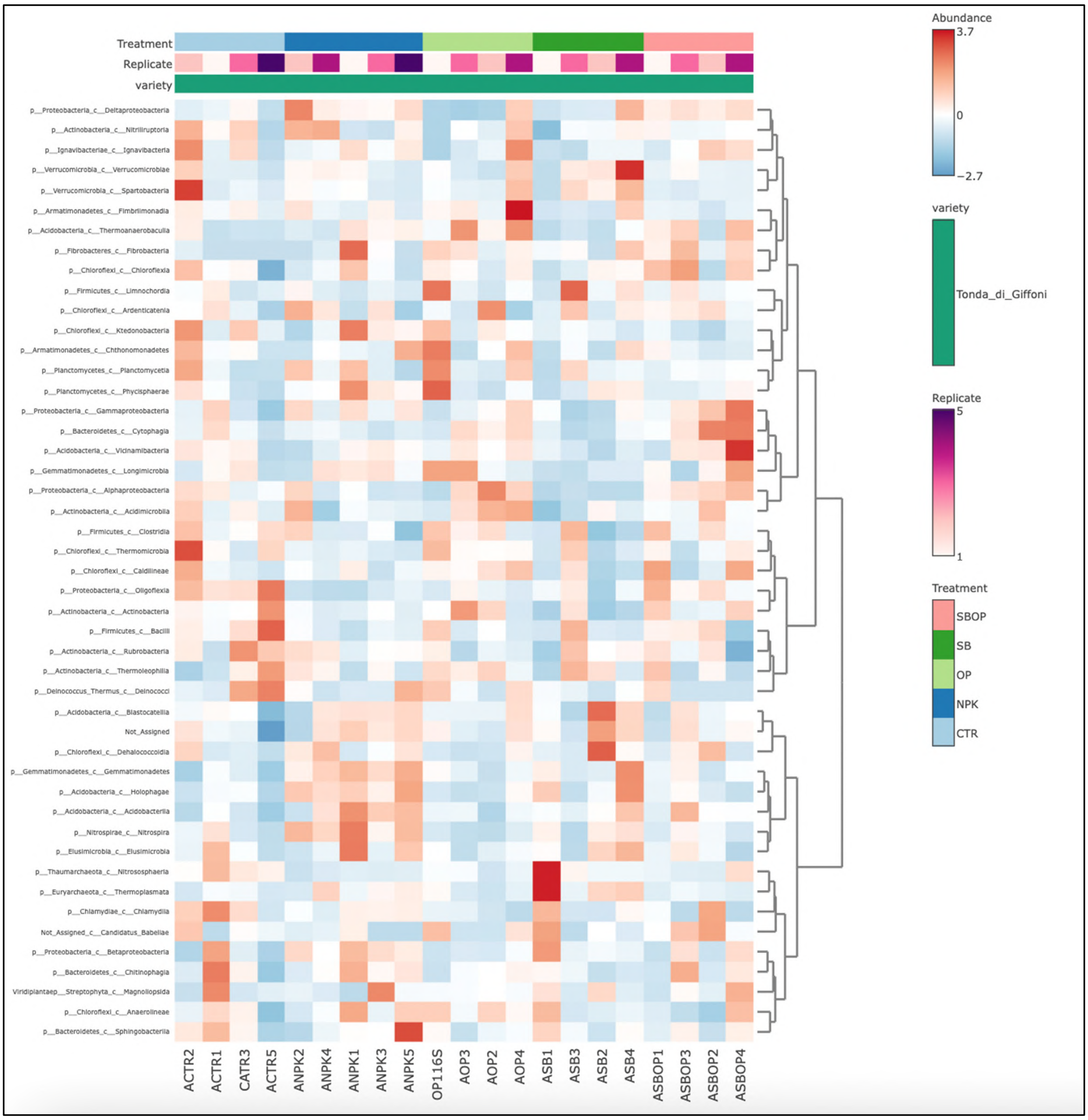


Figure 9. Bacteria heatmap of relative abundance at the class level.

A heatmap of fungi at the class level (Figure 10) shows that the SBOP treatment exhibits high abundance for several taxa, and in particular, this treatment could promote the growth of fungi belonging to these groups, suggesting a favorable environment for the proliferation of *Dothideomycetes* and *Eurotiomycetes*. The SB treatment shows a variable abundance pattern, with moderate *Saccharomycetes* and a higher abundance of *Sordariomycetes*. The effect of SB treatment varied between replicates, suggesting a less uniform response than other treatments. OP treatment generally shows low abundance for many taxa, especially in *Geoglossomycetes* and *Leotiomycetes.* The NPK treatment shows considerable variation in the abundance of taxa: a high abundance of *Orbiliomycetes* and a low abundance of *Sordariomycetes*. The CTR treatment shows a generally lower abundance for several taxa: *Agaricomycetes* and *Trebouxiophyceae*, compared to OP and SBOP. The dendrogram shows how taxa are grouped according to their abundance. The taxa *Dothideomycetes Eurotiomycetes* tend to cluster together, indicating a similar response to treatments.

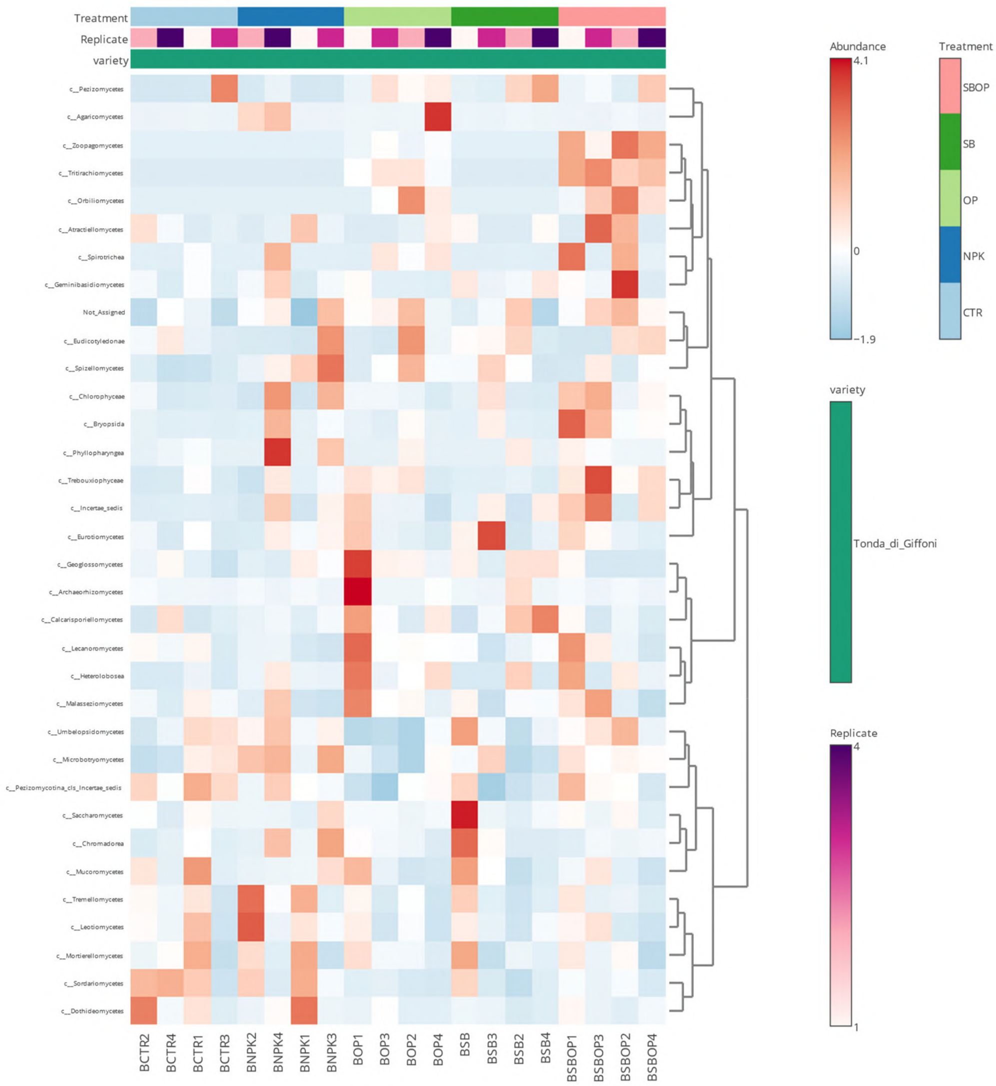


Figure 10. Fungi heatmap of relative abundance at the class level.

4. Discussion

The combined application of chemical and organic fertilizers has previously been documented to offer several advantages over synthetic fertilizers, including a balanced nutrient supply and a reduction in the environmental risks associated with excessive usage [40,41]. While some studies [42–44] suggest that short-term effects from applying organic fertilizers may not significantly impact microbial richness due to competition between microbes in bio-organic fertilizers and the existing local bacterial community, others present a contrasting view. For instance, Tian et al. [44] reported a decrease in soil bacterial diversity following organic fertilizer application, whereas other studies [45,46] highlighted the potential of organic fertilizers to enhance the soil microbiome, promoting bacterial growth and increasing population diversity.

Our study demonstrated that the combined fertilizer treatment, composed of organic, composted olive pomace (including a manure component) and sulfur and bentonite minerals, enhanced the microbial abundance in a similar manner to organic fertilizer (OP), rather than with sulfur-bentonite fertilizer (SB) alone.

Our research indicates that adding just 5% of organic material to sulfur-based mineral fertilizer can leverage beneficial increases in various diverse bacteria and fungi, offering a potential improvement in soil quality.

This observation agrees with the study conducted by Marra et al. [47] who demonstrated that bacterial communities were influenced by a sulfur-bentonite-based fertilizer combined with organic components (orange waste) in alkaline soils, at the same application rates used in our study, whilst increases in fungal communities were correlated with an organic fertilizer (horse manure), and actinomycetes were linked to NPK treatments. A recent study by Maffia et al. [48] further showed that compost derived from olive pomace, similar in composition to the one used in this study, significantly increased the number of actinomycete, fungal, and other bacterial taxa compared to the control and other treatments. However, these studies relied on traditional microbial counting methods, which have inherent limitations, such as selective culturing and under-representation of non-culturable microbes. Despite the positive increase in microbial abundance/diversity with 5% organic matter, the optimal fertilization rate at which to achieve maximum increases still requires further experimental verification. For example, Han et al. [49] found that 10–30% organic fertilizer significantly increased bacterial diversity in maize, while others argue for a higher percentage [50].

4.1. Effect of Fertilization on Microbial Composition and Biodiversity

At the phylum level, we observed that *Actinobacteria* and *Proteobacteria* were the dominant bacterial groups in the rhizosphere of *Corylus avellana* across all treatments. The increased abundance of *Actinobacteria* in the olive pomace compost (OP) treatment is noteworthy, as this bacterial phylum plays a key role in soil organic matter turnover and the breakdown of complex molecules such as cellulose and polycyclic aromatic hydrocarbons [51,52]. Similarly, the increased presence of *Proteobacteria* in the SBOP and OP treatments may be explained by the rise in soil carbon content following the application of organic amendments, as observed in previous studies [53]. As α-, β-, and γ-*Proteobacteria* are classified as "copiotrophs" that utilize labile carbon for growth, they tend to thrive in nutrient-rich environments [54,55], such as those in the OP and SBOP treatments.

Interestingly, an increase in *Acidobacteria* was also observed across all treatments, particularly in the SB treatment. According to Kalam et al. [56], these bacteria possess genes that enable them to survive in and competitively colonize the rhizosphere, fostering beneficial relationships with plants. Additionally, *Acidobacteria* are equipped with genes that enable them to metabolize both inorganic and organic nitrogen sources, effectively reducing nitrates, nitrites, and nitric oxide [57].

While *Firmicutes* were identified as the fifth most abundant phylum, the genus *Bacillus*—a member of *Firmicutes*—was the most dominant genus across treatments. *Bacillus* is a highly adaptable bacterium capable of surviving adverse environmental conditions by forming spores and degrading organic materials such as cellulose. Though *Firmicutes* as a whole may be less abundant relative to other phyla, the dominance of *Bacillus* is consistent with its ability to thrive in diverse soil environments [58]. However, while several studies have shown that *Bacillus* abundance increases with the application of organic fertilizers [59,60], our results align with Wu J et al. [61], who observed a sharp decline in *Bacillus* relative abundance in fertilized soils compared to unfertilized soils.

Regarding fungi, *Ascomycota* and *Streptophyta* were the dominant phyla in the rhizosphere of *Corylus avellana* across all treatments. Our study showed a lower relative abundance of *Ascomycota* in most treatments compared to the control (CTR). This finding is in line with a recent study by Sivojienė et al. [62], which also noted a reduction in the abundance of *Ascomycota* following organic fertilizer application, such as poultry manure. This could have been attributed to the more efficient competition for resources by other microorganisms or their better adaptation to new soil chemical conditions, such as fungi from the genus *Talaromyces*. This genus was particularly abundant in the OP treatment. A similar study reported a negative correlation between *Basidiomycota* abundance and high levels of total nitrogen (TN), soil organic carbon (SOC), and soil moisture (SM), but a positive correlation with aromatic substance availability (SA) [63]. This suggests that *Basidiomycota* may thrive in soils with high carbon and nitrogen content but where aromatic substances are more prevalent, as is often the case with organic fertilizers.

The second most abundant fungal phylum was *Streptophyta*, a group of land plants and algae that may compete with crops for nitrogen [64–66], potentially coming from contamination of the water used for this field experiment. Considering the competitive role of *Streptophyta* in nutrient uptake, particularly nitrogen, the SB treatment was observed to be associated with the smallest increase in *Streptophyta* abundance (25%) so could, therefore, be considered the most favorable treatment for minimizing competition between crops and this phylum.

Fungi belonging to the genus *Talaromyces* were notable for their role as primary decomposers of plant residues and as antagonists towards other fungi [67]. Some species of *Talaromyces* secrete organic acids and phosphatase, aiding in the dissolution of inorganic calcium phosphate and phosphate ester, which promotes phosphorus uptake by plants [68,69]. In our study, the SB treatment demonstrated the highest response in *Talaromyces* abundance compared to the control. This may have been due to the soil acidification induced by sulfur, which creates a favorable environment for *Talaromyces*; however, additional assessments of soil pH would be required to verify this. Additionally, bentonite present in SB treatments could have contributed to improvements in soil structure, moisture retention, and increases in phosphorus availability, thereby enhancing the growth-promoting effects of *Talaromyces* on plants.

The increase in alpha-diversity, particularly bacterial species richness as measured by the Chao1 index, in soils treated with sulfur bentonite (SB) and organic sulfur bentonite (SBOP), is consistent with the findings of Damo et al. [70], who reported that sulfur applications significantly increased both microbial abundance and diversity compared to sulfur-free treatments. Notably, when comparing SB and SBOP, the organic component of compost in the SBOP treatment plays a critical role in further enhancing species richness for both bacteria and fungi, even in short-term treatments. This observation aligns with other studies [71,72], which emphasize the pivotal role of organic matter in fostering microbial biodiversity.

Alpha diversity, as quantified through the Chao1 index, reflects the richness or the number of distinct species in the soil microbial community. Our results show that the inclusion of even a small percentage (5%) of organic material in the SBOP treatment resulted in a significant increase in fungal richness compared to the control (CTR), underscoring the importance of organic components in soil biodiversity. These results corroborate the findings of Hu et al. [43], who demonstrated that the use of organic fertilizers can significantly enhance microbial composition even after short-term application. This suggests that the composition and quality of organic matter are key factors in promoting soil alpha-diversity, a conclusion also supported by Guo et al. [73].

In terms of beta-diversity, which refers to the differences in community composition between treatments, the Bray–Curtis dissimilarity index highlighted the strong influence of fertilizer type on microbial community structure. Organic amendments, such as compost (OP) or sulfur bentonite, supported the establishment of distinct microbial communities compared to mineral fertilizers like NPK. This differentiation suggests that the organic matter, in combination with other components like sulfur, significantly reshapes soil microbial populations, which is a phenomenon widely observed in soil ecology [43]. However, the high variability within treatment groups, as if often seen in complex soil environments, may obscure clear clustering patterns, which can be influenced by several factors, including nutrient availability, microbial interactions, and soil structure.

This variability in microbial response suggests a nuanced interaction between the fertilizer types and the inherent soil microbial community, where the specific components of the organic matter (e.g., compost) play a critical role in driving both alpha and beta-diversity changes.

4.2. Sulfur with Organic Matter Enhances Beneficial Microbial Component

The results obtained with LEfSe (linear discriminant analysis effect size) analysis showed significant differences in bacterial and fungal communities between different fertilization treatments, allowing us to identify key taxa that influence soil health and plant-soil interactions. In particular, fertilizers containing organic components, such as the OP and SBOP, favored the proliferation of bacteria and fungi closely linked to the nutrient cycle, improving nutritional efficiency and soil quality.

Among bacteria, *Thiobacillus* was found to have a significantly higher relative abundance in SBOP-treated soils, emphasizing the critical role of this genus in the sulfur cycle. *Thiobacillus* oxidises the elemental sulfur and reduced compounds such as H^2S and thiosulphates, converting them to sulphate ($SO_4{}^{2-}$), a process that not only enriches the soil with essential sulphates, but also facilitates the solubilization of other nutrients, including phosphates and micronutrient metals [74]. This microbial activity promoted by the organic component of sulfur fertilizer highlights how the incorporation of organic materials can enhance the bioavailability of key nutrients.

Pseudoxanthomonas, another bacterial genus found with significant abundance in OP and SBOP treatments, contributes to the decomposition of complex organic compounds, such as lignin and cellulose, improving nutrient availability to plants. The presence of this genus has also been associated with an increase in soil biodiversity, with potential positive effects on rhizosphere health [75,76]. Along with bacteria such as *Pseudoxanthomonas*, fungi such as *Thermomyces* were also found to be more abundant in SBOP treatments. *Thermomyces* are crucial for the degradation of lignocellulose and facilitate the mineralization of organic matter, supporting the carbon and nitrogen cycle in the soil [77,78]. This synergy between bacteria and fungi creates a nutrient-rich environment that supports plant growth and health.

Bacteria belonging to the genus *Reyranella*, present in SBOP and OP treatments, play a significant role in carbon transformation and stabilization of soil microbial communities, contributing to soil resilience and health. This genus is known for its ability to adapt to variable soil conditions and interact positively with other microorganisms, improving the stability of microbial communities and promoting effective biogeochemical cycles. In parallel, the thermophilic fungus *Pseudallescheria* has been found in abundance with the addition of organic fertilizers, especially with SBOP, playing an important role in mineralizing organic matter and improving soil fertility [79,80]. This ability to decompose organic matter makes *Pseudallescheria* a key player in the nutrient cycle, particularly in agricultural systems using organic fertilizers.

The genus *Phaselicystis* was also present in abundance in the OP and SBOP treatments, and is a crucial component linking soil nutrient cycling and plant defense. This genus, and in particular the only known species *Phaselicystis flava*, contributes to the degradation of complex organic compounds, improving the availability of nutrients such as organic carbon and nitrogen, which are essential for plant growth support [81]. However, *Phaselicystis* also stands out for its role in plant defense due to its ability to produce arachidonic acid, an allelopathic metabolite that mediates interactions between microorganisms in the soil, particularly between arbuscular mycorrhizal fungi and bacteria [82]. Arachidonic acid not only modulates microbial interactions, but also recruits beneficial microorganisms into the rhizosphere, promoting nutrient turnover and protecting plants from abiotic and biotic stresses [82,83].

Next to bacteria, many fungi also play a crucial role in plant defense. *Tritirachium*, a fungus abundant in OP and SBOP treatments, is known for its entomopathogenic properties and protease production, which contribute to plant protection from pest attacks [84]. This highlights how the organic component of fertilizers, in addition to improving nutrition, promotes the growth of microorganisms with defensive properties.

Dactylella, another fungus present exclusively in OP and SBOP treatments, is known for its ability to trap nematodes and for its use in the biocontrol of fungal pathogens, contributing to a synergistic approach in natural plant protection. Its presence suggests a reduction in the need for external chemical inputs, improving soil resilience and promoting more sustainable agriculture [85]. Finally, the fungus *Chaetomium*, known to produce numerous metabolites with antifungal and photoprotective activity, showed a decrease in all treatments compared to the control, especially with OP. This could indicate that enriching the soil with treatments that promote beneficial microorganisms reduces the need for the protective action *Chaetomium* provides in untreated soil, highlighting the complexity of microbial interactions in an organic fertilization context [86,87].

5. Conclusions

The present study demonstrates that the combined application of chemical and organic fertilizers—specifically, an innovative blend of composted olive pomace and mineral constituents such as sulfur and bentonite—represents a promising strategy for enhancing soil biodiversity. Our findings indicate that even a modest addition of organic matter (5%) to sulfur-based fertilizers can significantly enhance both bacterial and fungal diversity, yielding effects comparable to those observed with purely organic fertilizers. These results underscore the potential of mixed fertilization strategies to sustain soil health and promote plant growth by optimizing microbial diversity and functionality. Notably, we observed substantial changes in the rhizosphere microbiome of *Corylus avellana* following the application of various fertilizers, which influenced microbiome composition. The application of organic fertilizers, including composted olive pomace and a sulfur-bentonite-olive pomace combination, notably improved the Chao1 index for bacterial and fungal communities. For example, the SBOP treatment showed an increase in Chao1 index compared to the control of 18% for bacterial communities and 22% for fungal communities compared to the unfertilized soil (CTR). The OP treatment, on the other hand, showed an increase of 16% for bacteria and 20% for fungi compared to the unfertilized soil (CTR). It is crucial to emphasize that our assessments focus on the short-term effects of these fertilization strategies. Future research will continue to explore the long-term implications of these practices on soil health and sustainability. Nonetheless, the current findings are promising, indicating positive outcomes for agricultural sustainability and the effective use of fertilizers, thereby contributing to a more balanced and sustainable approach to soil management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/life14121633/s1, Table S1: Bacteria Beta diversity Bray–Curtis. The table below summarizes the result of pairwise PERMANOVA analysis. The multi-testing adjustment is based on Benjamini–Hochberg procedure (FDR); Table S2: Fungi Beta diversity Bray–Curtis. The table below summarizes the result of pairwise PERMANOVA analysis. The multi-testing adjustment is based on Benjamini–Hochberg procedure (FDR); Table S3: Bacteria LEfSe results with a *p* value cut of 0.05 and Log LDA score of 2.0. The table below shows the 44 features ranked by their *p* values that are statistically significant; Table S4: Fungi LEfSe results with a *p* value cut of 0.05 and a Log LDA score of 2.0. The table below shows the 44 features ranked by their *p* values that are statistically significant.

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

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General Conclusion and Future Perspectives

This PhD thesis has addressed the urgent need for sustainable agricultural practices by developing and evaluating fertilizers derived from agro-industrial wastes. The research has demonstrated that these waste-derived fertilizers, rich in organic and mineral components, contribute significantly to circular economy initiatives, enhance soil fertility, promote microbial biodiversity, improve crop quality, and reduce environmental impacts.

A central focus of this study has been the role of organic fertilizers produced from agro-industrial wastes in improving the physical, chemical, and biological properties of soil. By systematically comparing different waste transformation techniques—including composting, anaerobic digestion, and crude waste pelleting—applied to organic waste types such as olive pomace and orange waste, the research has revealed that the choice of transformation method significantly influences the chemical composition and agronomic benefits of the resulting fertilizers. Each method offers distinct advantages for soil health, underscoring the importance of selecting the appropriate processing technique based on specific soil and crop requirements.

The study has shown that all tested fertilizers positively influence key soil parameters, including microbial biomass, enzymatic activity, and cation exchange capacity (CEC). Among the transformation methods, aerobic composting has emerged as the most effective, yielding fertilizers with superior organic carbon content, enhanced enzymatic activity, and improved CEC. Notably, composts derived from olive pomace and orange waste have demonstrated remarkable improvements in soil fertility, particularly by stimulating essential soil enzymes such as fluorescein diacetate hydrolase, dehydrogenase, and microbial biomass carbon. These findings provide compelling evidence that fertilizers derived from agro-industrial wastes—especially those produced through aerobic composting—offer a sustainable and efficient solution for improving soil quality, supporting active soil life, and fostering resilient agricultural systems.

Furthermore, the integration of orange waste or olive pomace with sulfur and bentonite has proven highly effective in formulating fertilizers tailored to address the characteristic alkalinity of Calabrian soils. This approach has enhanced the bioavailability of essential macro- and micronutrients while significantly enriching soil organic matter content. The results clearly indicate that sulfur-based fertilizers not only improve soil quality but also surpass commonly used organic and inorganic fertilizers in terms of efficacy. Additionally, the research has identified optimal application rates for sulfur-bentonite-based fertilizers, ensuring significant improvements in critical soil chemical and biological properties while mitigating the risk of excessive soil acidification. These findings highlighted the potential of these fertilizers in promoting balanced soil pH management and sustaining healthier, more productive soils.

In conclusion, this study reinforced the viability of agro-industrial waste-derived fertilizers as an effective and sustainable alternative to conventional fertilizers. By leveraging waste transformation technologies, these fertilizers not only contribute to soil health and productivity but also support broader environmental and economic sustainability goals. Their adoption in agricultural practices could play a crucial role in advancing more circular, resilient, and sustainable food production systems.

The impact of fertilizers derived from agro-industrial wastes on soil biodiversity is particularly noteworthy. Metagenomic analysis of the rhizosphere has revealed that the combined application of sulfur and bentonite with an organic component, such as olive pomace compost, represents a promising strategy for enhancing microbial diversity. Even a modest addition of organic matter (5%) to sulfur-based fertilizers can significantly improve both bacterial and fungal diversity, producing effects comparable to those observed with purely organic fertilizers. This improvement fosters the growth and activity of beneficial microorganisms, which play a crucial role in soil health and plant development.

Beyond soil biodiversity, the positive influence of these eco-sustainable fertilizers on crop quality constitutes a second key pillar of this research. Studies conducted on tomatoes and garlic have demonstrated that these fertilizers not only support plant growth but also enhance the concentration of bioactive compounds, including phenols, flavonoids, and vitamins. Since these compounds are essential for human health, their enrichment adds significant value to agricultural products, suggesting a potential role for these fertilizers in both agronomic and nutraceutical sectors. Notably, the observed bio stimulant effect in tomatoes highlights how specific formulations, particularly those incorporating orange waste with sulfur and bentonite, can enhance both the nutritional and organoleptic properties of the produce, ultimately benefiting both growers and consumers.

From an environmental perspective, fertilizers produced from agro-industrial wastes represent a sustainable alternative to conventional fertilization practices, aligning with the principles of circular economy and resource efficiency. Life Cycle Assessments (LCA) conducted on these fertilizers have demonstrated their significantly lower environmental impact compared to both synthetic and commercial organic fertilizers. An in-depth analysis of industrial tomato production, comparing different fertilization techniques, including the novel orange waste–

based fertilizer with sulfur and bentonite, a commercial synthetic fertilizer, and a commercial organic fertilizer, has shown that the eco-sustainable fertilizer yields the lowest carbon and water footprints.

Furthermore, within the olive oil production chain, the proportion of olive oil waste and the extraction methods used have a profound impact on compost quality and its environmental footprint across multiple impact categories. In particular, the research has highlighted significant reductions in eutrophication (kg PO_4^{3-} eq) and acidification (kg SO_2 eq) when utilizing optimized composting processes.

These findings further reinforced the environmental benefits of adopting waste-derived fertilizers, demonstrating their potential to mitigate ecological degradation while maintaining high agricultural productivity and providing strong evidence that fertilizers derived from agro-industrial wastes not only enhance soil biodiversity and crop quality but also contribute to reducing the environmental impact of agricultural systems. Their adoption can play a crucial role in promoting sustainable farming practices, advancing both ecological and economic sustainability in modern agriculture.

The findings of this research highlighted significant reductions in photochemical oxidation (kg C_2H_4 eq), abiotic depletion (MJ), and global warming potential (kg CO_2 eq), suggesting that adopting these sustainable fertilizers could play a crucial role in mitigating the environmental impact of agricultural practices, aligning with global efforts to combat climate change and promote resource efficiency.

The results of this thesis opened various avenues for further research and practical applications, particularly in enhancing the effectiveness, scalability, and broader applicability of these sustainable fertilizers. Future studies should focus on optimizing transformation processes for different types of agro-industrial wastes, tailoring fertilizer formulations to meet the specific nutrient and structural requirements of diverse crops and soil types. Establishing standardized protocols would be crucial to ensuring the consistency, efficiency, and reliability of waste-derived fertilizers, allowing them to meet stringent agronomic and environmental standards while facilitating their widespread adoption. While this research provided strong evidence of the short-term benefits of these fertilizers, long-term studies would be essential to fully assess their impact on soil health, biodiversity, and crop productivity over multiple growing seasons. Such investigations would offer deeper insights into how these fertilizers influence soil microbiomes, nutrient cycling, and overall ecosystem stability, ultimately paving the way for their large-scale implementation in modern agriculture.

Beyond scientific advancements, the successful integration of sustainable fertilizers into conventional agricultural systems would also depend on supportive policies that encourage waste valorization and reduce reliance on synthetic fertilizers. Although European policies have increasingly promoted circular economy initiatives, the lack of comprehensive reference standards remained a key barrier to widespread adoption. Developing clear regulatory frameworks would be critical to facilitating sustainability assessments that account for both soil quality and environmental impact. Transparent and rigorous evaluation methodologies would not only strengthen the scientific credibility of waste-derived fertilizers but also drive a global transition toward more sustainable and resilient agricultural systems.

In summury, this research underscored the transformative potential of fertilizers derived from agro-industrial wastes, demonstrating their ability to enhance soil health, biodiversity, and crop quality while reducing environmental footprints. By bridging the gap between scientific innovation, agricultural practice, and policy development, these fertilizers could contribute to a more sustainable, circular, and climate-resilient agricultural sector, offering a viable path toward a greener and more efficient future in food production.

List of Figures

Chapter 1 - Introduction

Chapter 2 - *Recycling of agricultural (orange and olive) bio-wastes into ecofriendly fertilizers for improving soil and garlic quality*

Chapter 3 - *Effects of fertilizer produced from agro-industrial wastes on the quality of two different soils*

Chapter 7 - *Transforming Agricultural and Sulfur Wastes into Fertilizer: Assessing Short-Term Effects on Microbial Biodiversity via a Metagenomic Approach*

List of Tables

Chapter 5- *Waste-Derived Fertilizer Acts as Biostimulant, Boosting Tomato Quality and Aroma*

APPENDIX 1- Additional papers

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], Adele Muscolo[1*].

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

*Correspondence: amuscolo@unirc.it

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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

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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.

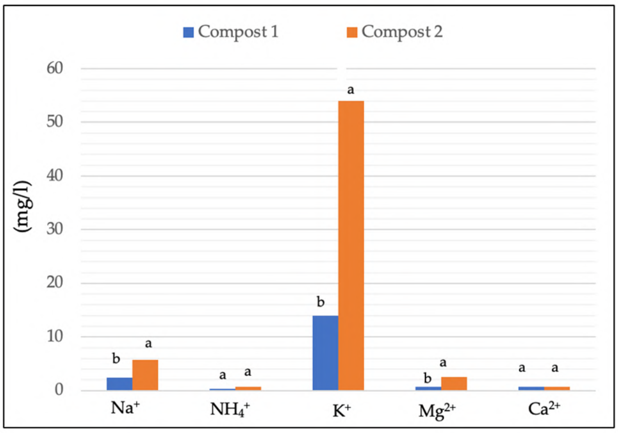
3. Results

3.1. Compost Properties

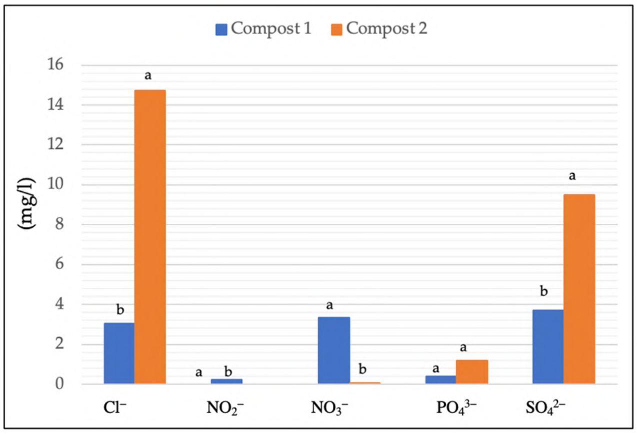
The composting procedure underwent three repetitions through independent experiments. The outcomes consistently revealed that each compost derived from these experiments exhibited identical chemical characteristics. This observation strongly indicated that the adopted procedure has been successfully 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 $p \leq 0.05$).

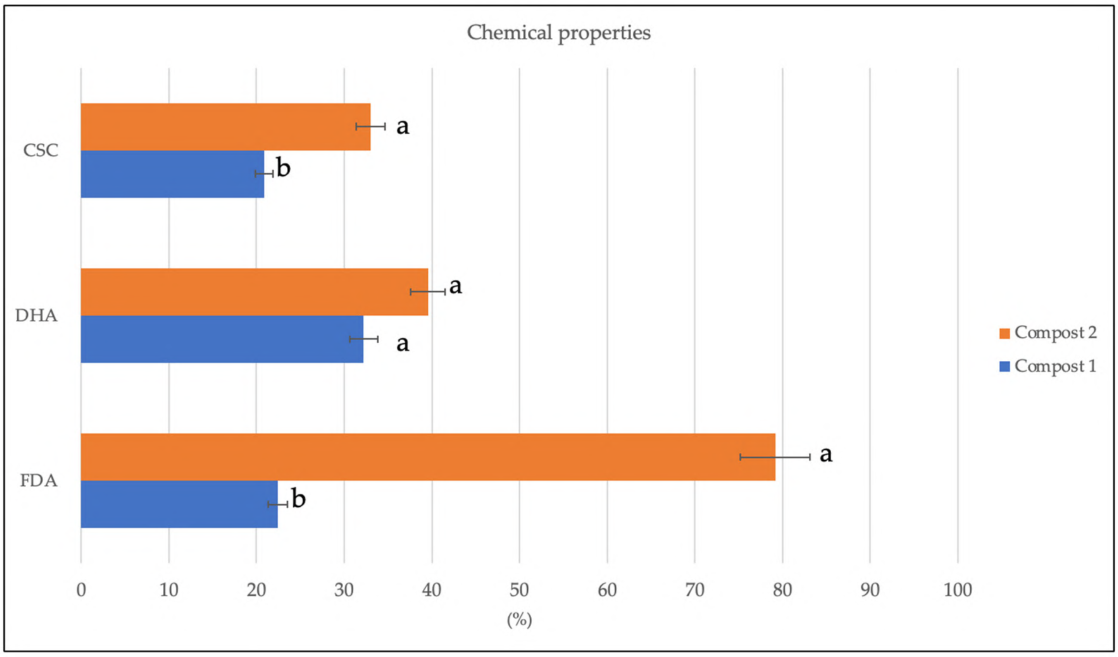


Figure 2. Fluorescein diacetate hydrolase (FDA, µg fluorescein g^{-1} d.w.), dehydrogenase (DHA, µg TTF g^{-1} h^{-1}d.w.), cation exchange capacity (CEC, $cmol^{(+)}$ Kg^{-1}) detected in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting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

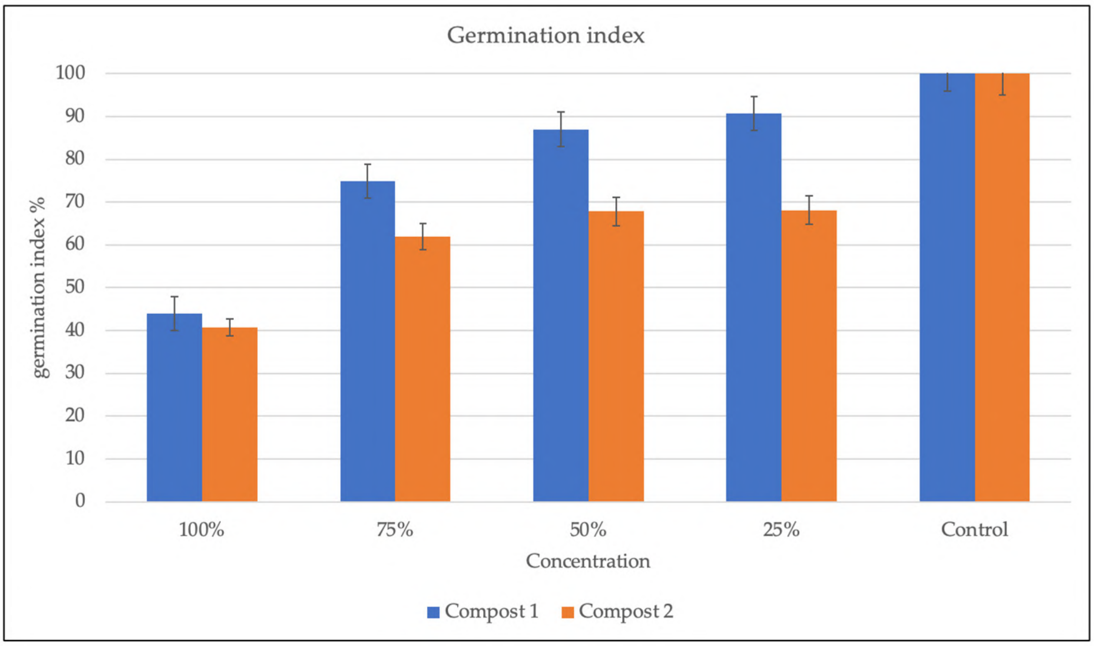


Figure 3. Germination index in Compost 1 (50% wood sawdust + 50% wet wastes) and Compost 2 (10% Straw + 90% wet wastes) 120 days after the composting process.

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} ± 0.15	8.09 ^{d} ± 0.76	12.60 ^{c} ± 0.23	53.58 ^{b} ± 0.24	93.95 ^{a} ± 1.4
C2	−3.26 ^{e} ± 0.2	88.11 ^{d} ± 0.6	109.51 ^{c} ± 0.2	243.50 ^{a} ± 0.4	212.88 ^{b} ± 1.3
ID	Cl^-	Cl^-	Cl^-	Cl^-	Cl^-
C1	−5.86 ^{e} ± 0.2	53.23 ^{d} ± 0.16	120.59 ^{c} ± 1.6	138.23 ^{b} ± 1.8	367.99 ^{a} ± 3.6
C2	−4.330 ^{e} ± 0.1	87.40 ^{d} ± 0.5	124.08 ^{c} ± 0.1	311.51 ^{b} ± 0.1	461.24 ^{a} ± 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**

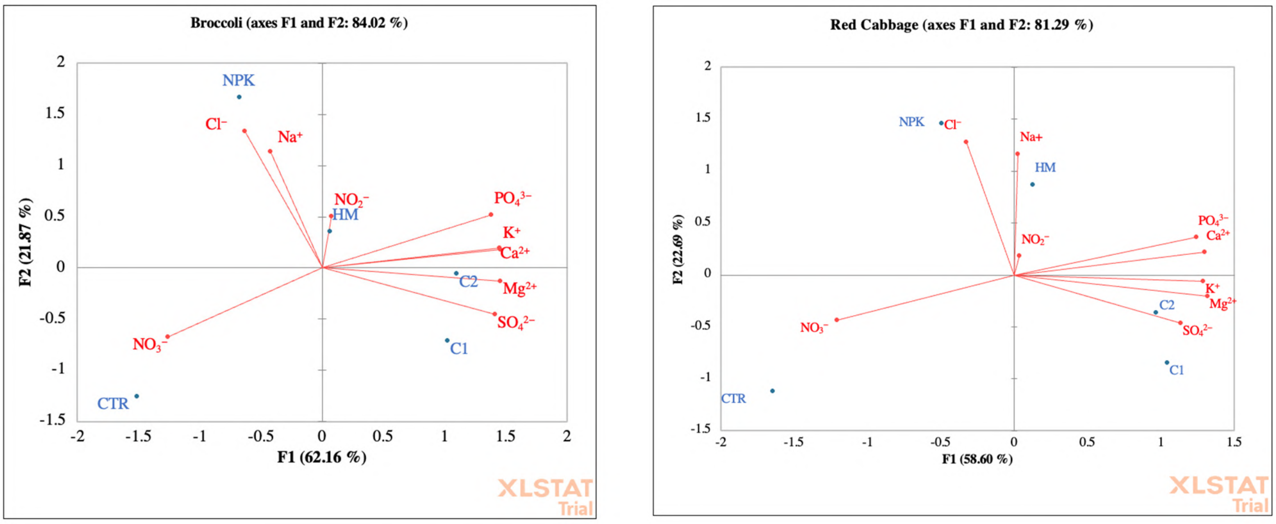
Table 6. Cations and anions concentrations (mg/L) detected 90 days after treatments with the different fertilizers. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw + 90% wet wastes Na^+ (sodium), K^+ (potassium), 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} ± 0.02	22.2 ^{b} ± 0.01	25.6 ^{a} ± 0.03	25.2 ^{a} ± 0.01	25.5 ^{a} ± 0.01
pH (H_2O)	8.45 ^{a} ± 0.12	8.46 ^{a} ± 0.02	8.47 ^{a} ± 0.05	8.44 ^{a} ± 0.05	8.41 ^{a} ± 0.01
pH (KCl)	7.1 ^{a} ± 0.07	7.01 ^{a} ± 0.06	6.99 ^{a} ± 0.05	6.94 ^{a} ± 0.04	6.97 ^{a} ± 0.05
EC	350 ^{c} ± 0.23	301 ^{c} ± 0.22	297 ^{c} ± 0.12	530 ^{b} ± 0.17	740 ^{a} ± 0.14
OC	1.78 ^{b} ± 0.19	1.69 ^{b} ± 0.22	2.13 ^{ab} ± 0.11	2.9 ^{a} ± 0.09	3.3 ^{a} ± 0.09
TN	0.19 ^{a} ± 0.17	0.23 ^{a} ± 0.09	0.21 ^{a} ± 0.13	0.19 ^{a} ± 0.12	0.20 ^{a} ± 0.11
C/N	9.4 ^{b} ± 0.15	7.39 ^{c} ± 0.15	19.1 ^{a} ± 0.16	15.2 ^{a} ± 0.11	16.5 ^{a} ± 0.14
WSP	282 ^{b} ± 0.32	320 ^{a} ± 0.52	315 ^{a} ± 0.42	138 ^{c} ± 1.12	170 ^{c} ± 0.92
MBC	433.3 ^{c} ± 0.52	733 ^{b} ± 0.17	798 ^{b} ± 0.42	897.33 ^{a} ± 0.52	961.4 ^{a} ± 0.32
FDA	5.14 ^{a} ± 0.44	5.44 ^{a} ± 0.33	5.33 ^{a} ± 0.27	4.88 ^{b} ± 0.36	4.81 ^{b} ± 0.18
DHA	20.1 ^{b} ± 0.72	22.1 ^{b} ± 0.32	24.1 ^{b} ± 0.42	32.92 ^{a} ± 0.32	38.09 ^{a} ± 0.42
BACT	1.3 × $10^{5\,c}$ ± 1.42	1.1 × $10^{5\,c}$ ± 2.12	1.6 × $10^{5\,c}$ ± 3.32	5 × $10^{5\,b}$ ± 3.13	8.3 × $10^{5\,a}$ ± 2.12
FUN	4.6 × $10^{4\,a}$ ± 3.12	4.5 × $10^{4\,a}$ ± 1.42	4.6 × $10^{4\,a}$ ± 2.62	2.7 × $10^{4\,b}$ ± 2.11	3 × 10^4 ± 2.02 ^{b}
ACT	5.7 × $10^{4\,a}$ ± 2.12	3.7 × $10^{4\,b}$ ± 4.12	6.7 × $10^{4\,a}$ ± 1.12	1.3 × $10^{5\,c}$ ± 3.16	1.5 × $10^{5\,c}$ ± 2.21
CEC	16 ^{b} ± 0.13	12 ^{c} ± 0.12	19 ^{ba} ± 0.18	22 ^{a} ± 0.11	22.9 ^{a} ± 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.

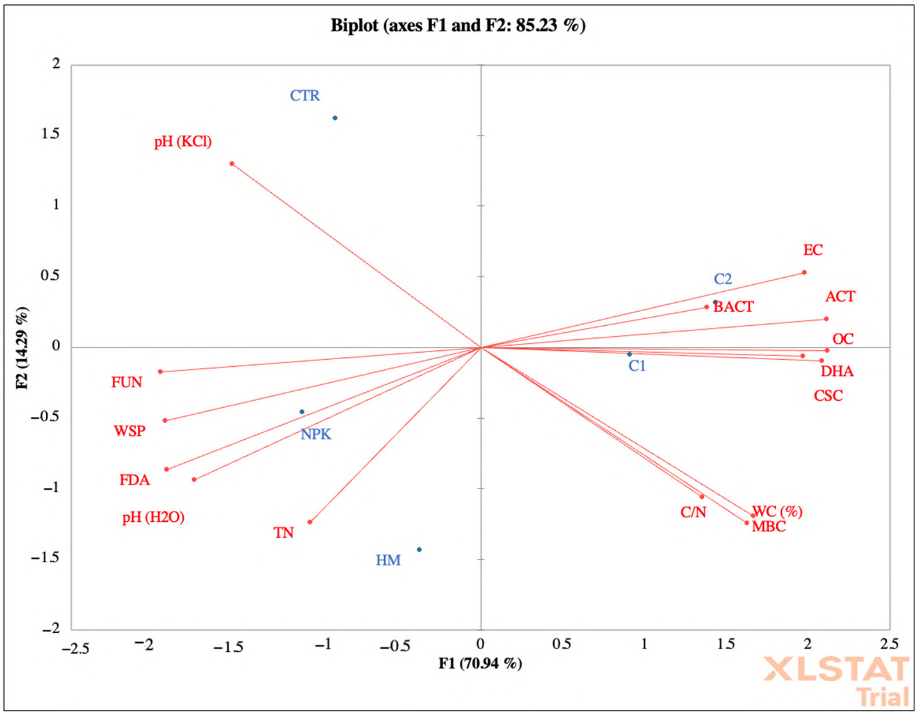


Figure 5. Principal component analyses of chemical and biochemical properties of soil located in Motta San Giovanni before fertilization. CTR (control) soil without fertilizer; NPK = nitrogen–phosphorous–potassium; HM = horse manure; C1 50% wood sawdust + 50% wet wastes, C2 10% straw and 90% wet wastes.

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.

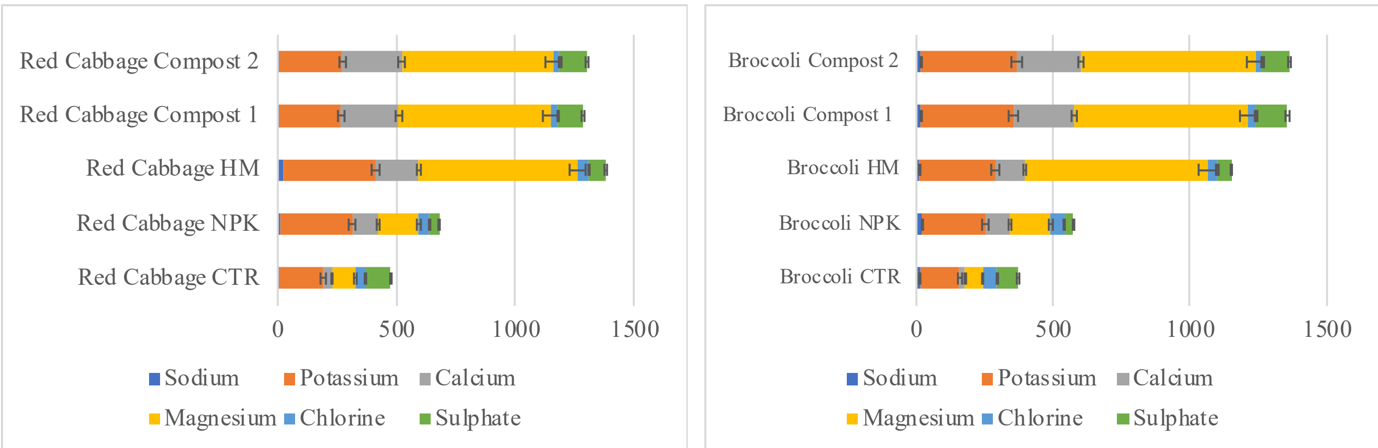
Broccoli Calabrese	**CTR**	**NPK**	**HM**	**C1**	**C2**
Chl a	114 ^{b} ± 0.43	120 ^{b} ± 0.12	142 ^{a} ± 0.02	158 ^{a} ± 0.12	167 ^{a} ± 0.19
Chl b	60 ^{a} ± 2.25	54 ^{a} ± 3.11	55 ^{a} ± 1.11	57 ^{a} ± 1.45	59 ^{a} ± 1.24
Chla/Chlb	1.9 ^{b} ± 1.11	2.2 ^{b} ± 1.12	2.58 ^{a} ± 1.54	2.77 ^{a} ± 1.12	2.83 ^{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).



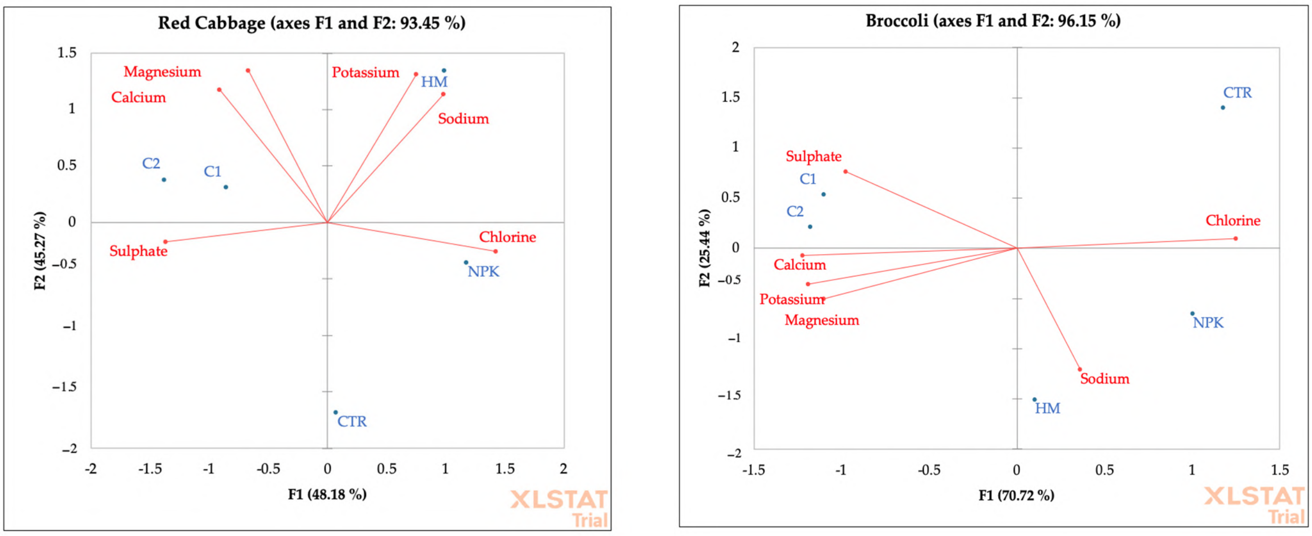
(**a**) (**b**)

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.

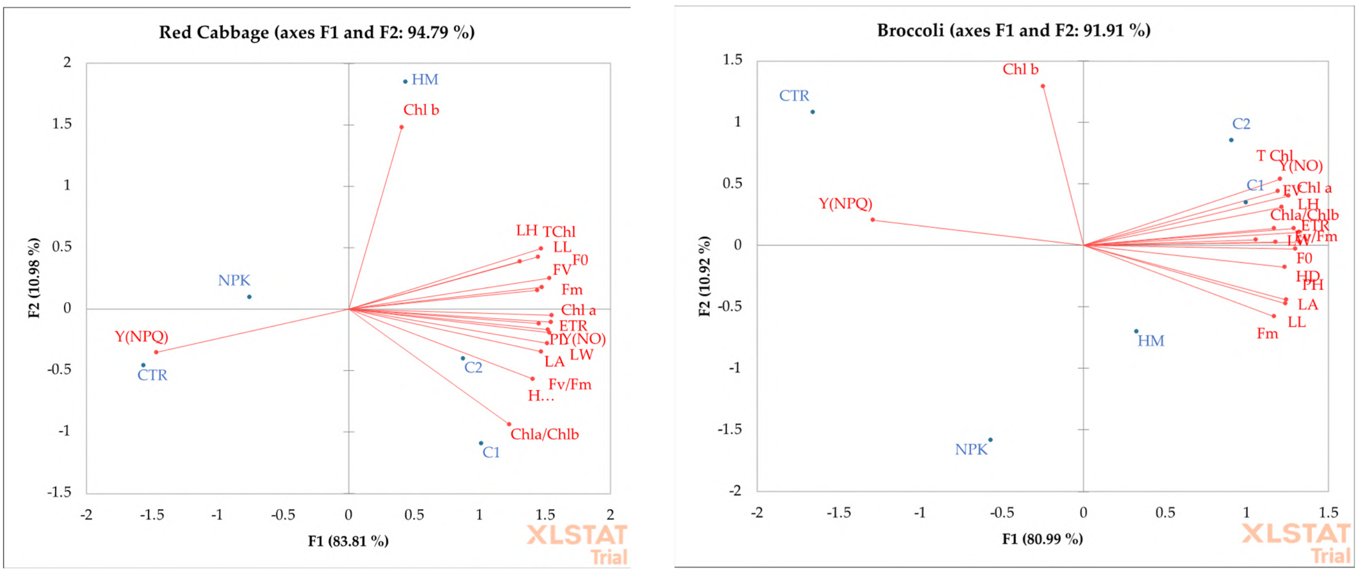
The best accumulation of ions has been observed in broccoli leaves treated with both composts (Figure 6).

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.

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Humic substances from Waste-Based Fertilizers from 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 author: amuscolo@unirc.it

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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

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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.

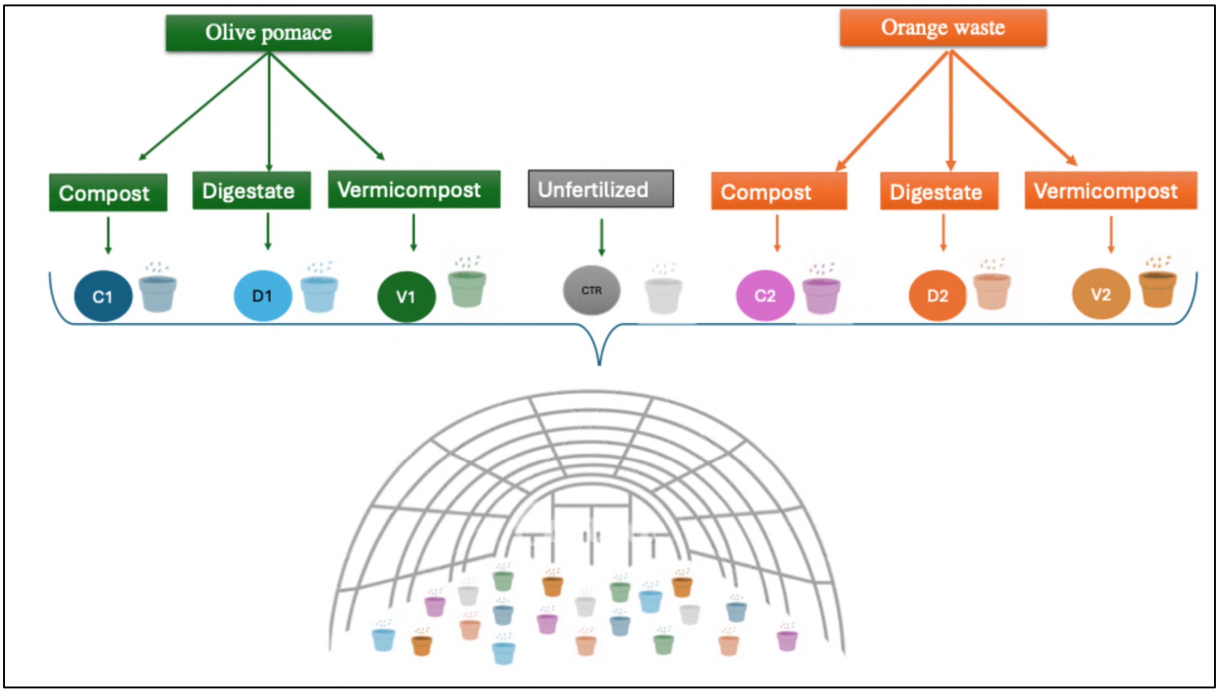

Figure 1. Experimental design in greenhouse. CTR unfertilized soil; C1 (90% wastes from olive oil + 10% straw), C2 (90% orange waste and 10% of straw), D1 (Digestate coming from olive waste 50%, and animal manure and maize silage 50%), D2 (Digestate coming from orange waste 50%, animal manure and maize 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} ± 0.05	7.6 ^{b} ± 0.5	7.49 ^{b} ± 0.03	7.61 ^{b} ± 0.02	8.5 ^{a} ± 0.2	8.3 ^{a} ± 0.8
BD (kg × m^{-3})	598 ^{c} ± 9.0	558 ^{c} ± 12	554 ^{b} ± 9	577 ^{b} ± 8	788 ^{a} ± 9.2	768 ^{a} ± 11
EC (mS × cm^{-1})	1.3 ^{b} ± 0.25	1.8 ^{b} ± 0.2	3.18 ^{a} ± 0.2	2.5a ^{b} ± 0.4	1.3 ^{b} ± 0.2	1.5 ^{b} ± 0.4
WC (%)	47 ^{b} ± 3.2	44 ^{b} ± 3	49.6 ^{b} ± 2	42.3 ^{b} ± 1	64 ^{a} ± 7	69 ^{a} ± 6
TC (%)	44 ^{b} ± 2.40	49 ^{b} ± 2.4	52.8 ^{a} ± 1.2	59.5 ^{a} ± 2	45 ^{b} ± 1.4	47 ^{b} ± 1.4
TN (%)	2.5 ^{ab} ± 0.22	2.7 ^{ab} ± 0.8	2.13 ^{b} ± 0.03	2.66 ^{ab} ± 0.02	3.7 ^{a} ± 0.2	2.2 ^{a} ± 0.9
C/N	17.6 ^{ab} ± 1.6	18.1 ^{ab} ± 1.6	19.19 ^{ab} ± 0.6	22.03 ^{a} ± 0.8	12.1 ^{b} ± 0.5	21.4 ^{a} ± 0.9
Na^+ (mg g^{-1} dw)	1.1 ^{c} ± 0.06	0.9 ^{c} ± 0.02	4.69 ^{a} ± 0.09	2.39 ^{b} ± 0.07	0.9 ^{c} ± 0.08	0.8 ^{c} ± 0.1
NH_4^+ (mg g^{-1} dw)	0.7 ^{b} ± 0.02	0.6 ^{b} ± 0.01	0.5 ^{a} ± 0.04	0.33 ^{b} ± 0.04	1.5 ^{a} ± 0.02	1.53 ^{a} ± 0.01
K^+ (mg g^{-1} dw)	17 ^{a} ± 1.50	18 ^{a} ± 1.3	7.57 ^{b} ± 0.2	9.65 ^{b} ± 0.3	0.5 ^{d} ± 0.02	3.6 ^{c} ± 0.5
Mg^{2+} (mg g^{-1} dw)	1.1a ^{b} ± 0.1	1.8 ^{a} ± 0.2	0.37 ^{b} ± 0.02	1.22 ^{ab} ± 0.04	0.53 ^{b} ± 0.08	0.85 ^{b} ± 0.06
Ca^{2+} (mg g^{-1} dw)	2.4 ^{a} ± 0.3	2.9 ^{a} ± 0.2	0.21 ^{b} ± 0.03	2.3 ^{a} ± 0.06	1.7 ^{a} ± 0.2	1.8 ^{a} ± 0.1
Cl^- (mg g^{-1} dw)	nd	nd	11.12 ^{a} ± 0.9	9.23 ^{a} ± 1.1	nd	0.48 ^{b} ± 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} ± 0.002	0.51 ^{a} ± 0.01	nd	0.87 ^{a} ± 1.1	0.21 ^{b} ± 0.03	0.42 ^{a} ± 0.02
PO_4^{3-} (mg g^{-1} dw)	0.43 ^{b} ± 0.03	0.90 ^{ab} ± 0.03	1.25 ^{a} ± 0.01	1.44 ^{a} ± 0.02	0.47 ^{b} ± 0.06	0.63 ^{b} ± 0.04
SO_4^{2-} (mg g^{-1} dw)	0.27 ^{b} ± 0.02	0.87 ^{ab} ± 0.02	0.93a ^{b} ± 0.02	1.33 ^{a} ± 0.02	0.11 ^{b} ± 0.01	0.44 ^{ab} ± 0.02
WSP (mg TAE g^{-1} dw)	2.42 ^{ab} ± 0.06	1.0 ^{b} ± 0.6	2.17 ^{b} ± 0.03	1.77 ^{b} ± 0.05	5.4 ^{a} ± 1	2.1 ^{b} ± 0.5
ON/TN	93 ^{a} ± 5	99 ^{a} ± 3	95.3 ^{a} ± 2	96.1 ^{a} ± 3	92 ^{a} ± 8	93 ^{a} ± 5
NH_4^+-N/NO_3^--N	1.66 ^{c} ± 0.13	1.17 ^{c} ± 0.13	nd	0.37 ^{c} ± 0.05	7.14 ^{a} ± 0.07	3.64 ^{b} ± 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} ± 1.4	49 ^{ab} ± 1.9	53 ^{a} ± 2.4	59 ^{ba} ± 1.9	45 ^{b} ± 1.5	47 ^{b} ± 1.2
TEC (%)	17 ^{b} ± 1	22 ^{a} ± 1.4	24 ^{a} ± 1.1	28 ^{a} ± 1.5	18 ^{b} ± 1	20 ^{ab} ± 1.5
HA + FA	11 ^{c} ± 1	18 ^{b} ± 1.6	19 ^{b} ± 1.5 ^{b}	25 ^{a} ± 1.4	11 ^{c} ± 1	12 ^{c} ± 1
HA	6 ^{c} ± 0.5	10 ^{a} ± 1.4	8 ^{b} ± 0.9	9 ^{ab} ± 0.9	9 ^{ab} ± 1	10 ^{a} ± 1
FA	5 ^{d} ± 0.3	8 ^{c} ± 0.4	11 ^{b} ± 1.0	16 ^{a} ± 1.1	2 ^{e} ± 0.2	2 ^{e} ± 0.1
HR (%)	25 ^{b} ± 1.8	36 ^{a} ± 1.5	35 ^{a} ± 1	39 ^{a} ± 1.5	24 ^{b} ± 1.4	26 ^{b} ± 1.5
HD (%)	64 ^{b} ± 1.9	80 ^{a} ± 2.9	81 ^{a} ± 2.5	87 ^{a} ± 2	61 ^{b} ± 1.9	63 ^{b} ± 2
HI (%)	0.57 ^{a} ± 0.01	0.25 ^{b} ± 0.04	0.22 ^{b} ± 0.04	0.15 ^{b} ± 0.03	0.63 ^{a} ± 0.06	0.58 ^{a} ± 0.05
E4/E6	7.3 ^{a} ± 0.9 ^{a}	4.5 ^{b} ± 0.6 ^{b}	6.1 ^{a} ± 0.8	4.2 ^{b} ± 0.7	9.3 ^{a} ± 1.0	7.1 ^{a} ± 0.9
TA (mol kg^{-1})	9.5 ^{ab} ± 0.8	8.9 ^{b} ± 0.4	10.1 ^{a} ± 0.5	11.4 ^{a} ± 0.6	6.5 ^{c} ± 0.8	6.9 ^{c} ± 0.8
OH (mol kg^{-1})	5.4 ^{a} ± 0.1	2.1 ^{b} ± 0.5	4.9 ^{a} ± 0.4	4.2 ^{a} ± 0.4	5.1 ^{a} ± 0.3	4.9 ^{a} ± 0.2
COOH (mol kg^{-1})	4.1 ^{b} ± 0.5	6.8 ^{a} ± 0.4	5.2 ^{ab} ± 0.6	7.4 ^{a} ± 0.3	1.4 ^{c} ± 0.5	2 ^{c} ± 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

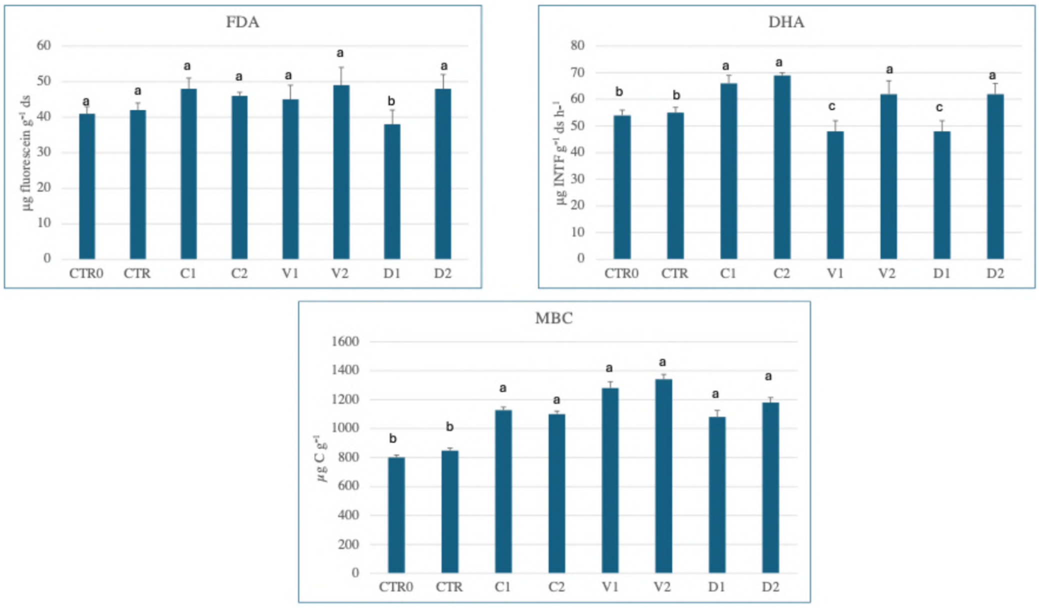

Figure 2. Fluorescein Hydrolase activity (FDA, µg fluorescein g^{-1} ds), Dehydrogenase activity (DHA, µg INTF g^{-1} ds h^{-1}), Microbial Biomass Carbon (MB µg C g^{-1} fs), of potted alkaline sandy-loam soils CTR0, and six months after the addition of C1, C2, D1, D2, V1, V2. Not amended soil was used 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).

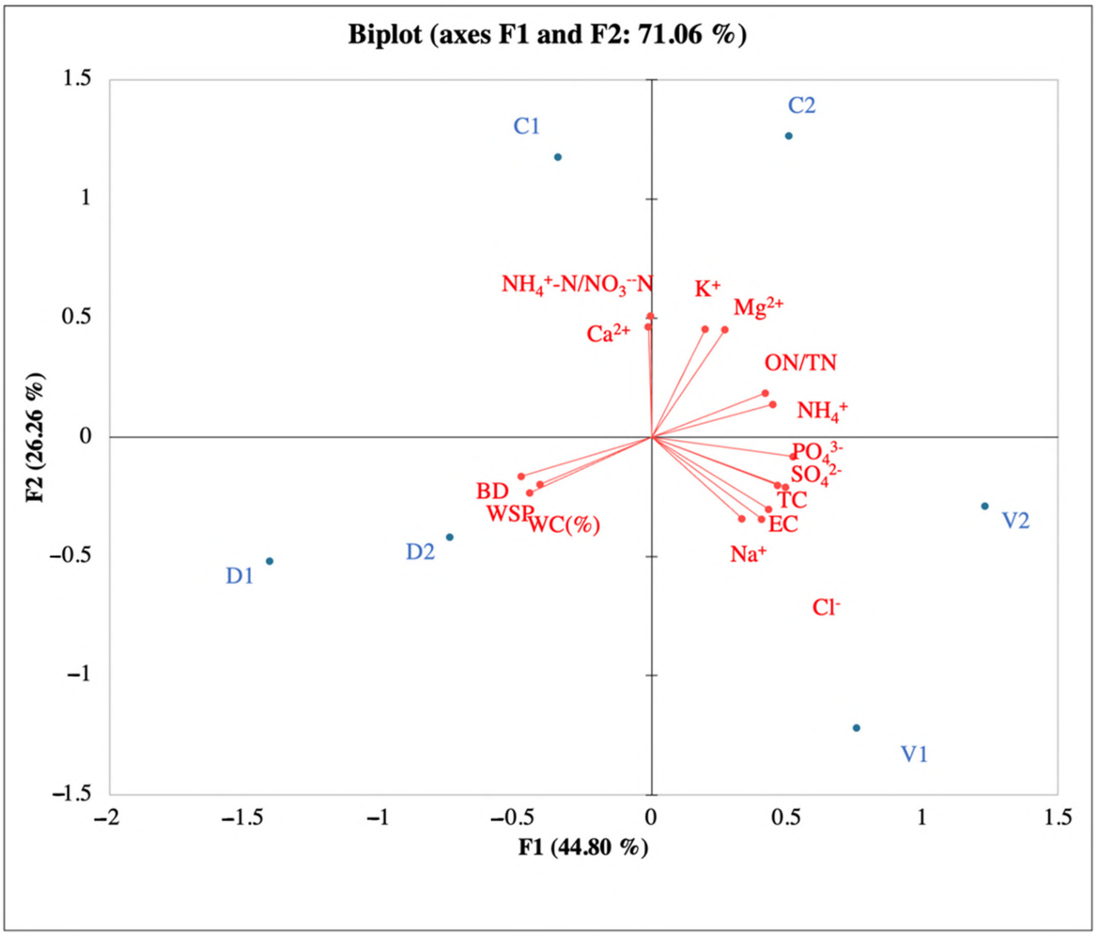

Figure 3. PCA of chemical characteristic of: C1, C2, D1, D2, V1 and V2.

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**

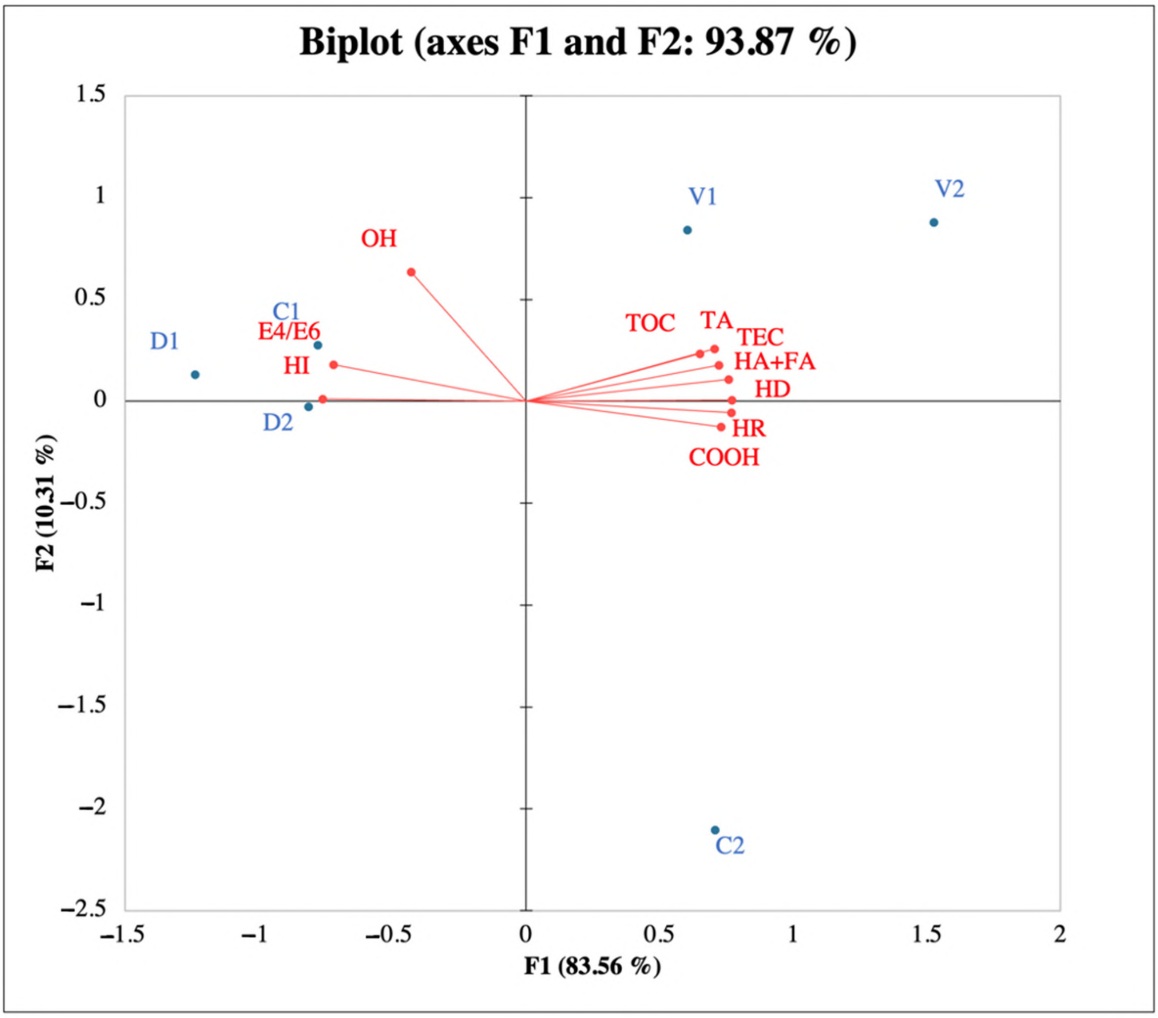

Figure 4. PCA of humic characteristics of C1, C2, D1, D2, V1 and V2.

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.

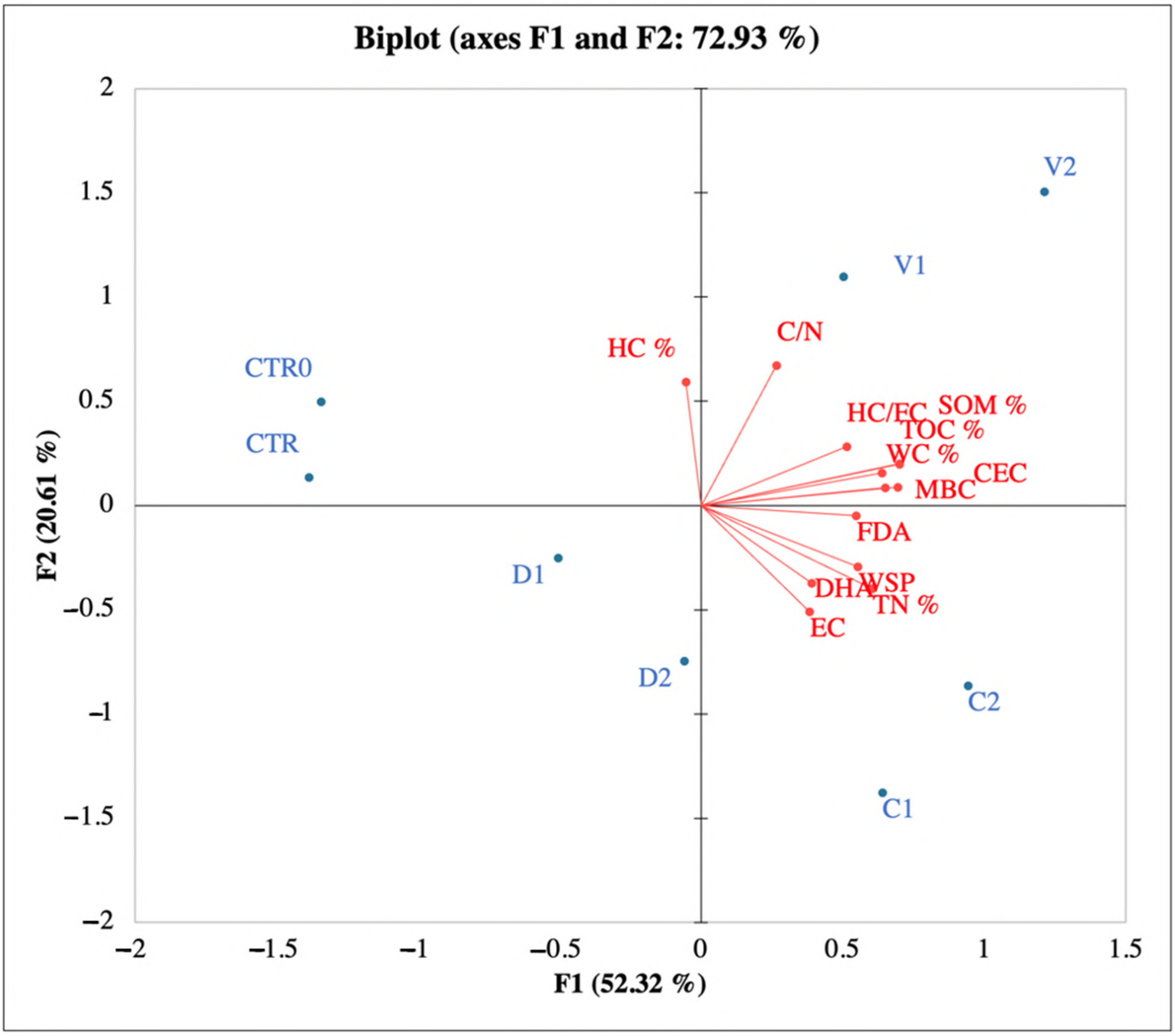

Figure 5. PCA of physical and chemical properties of potted alkaline sandy-loam soils CTR0, not amended soil was used as control (CTR) and six months after the addition of: C1, C2, D1, D2, V1 and V2.

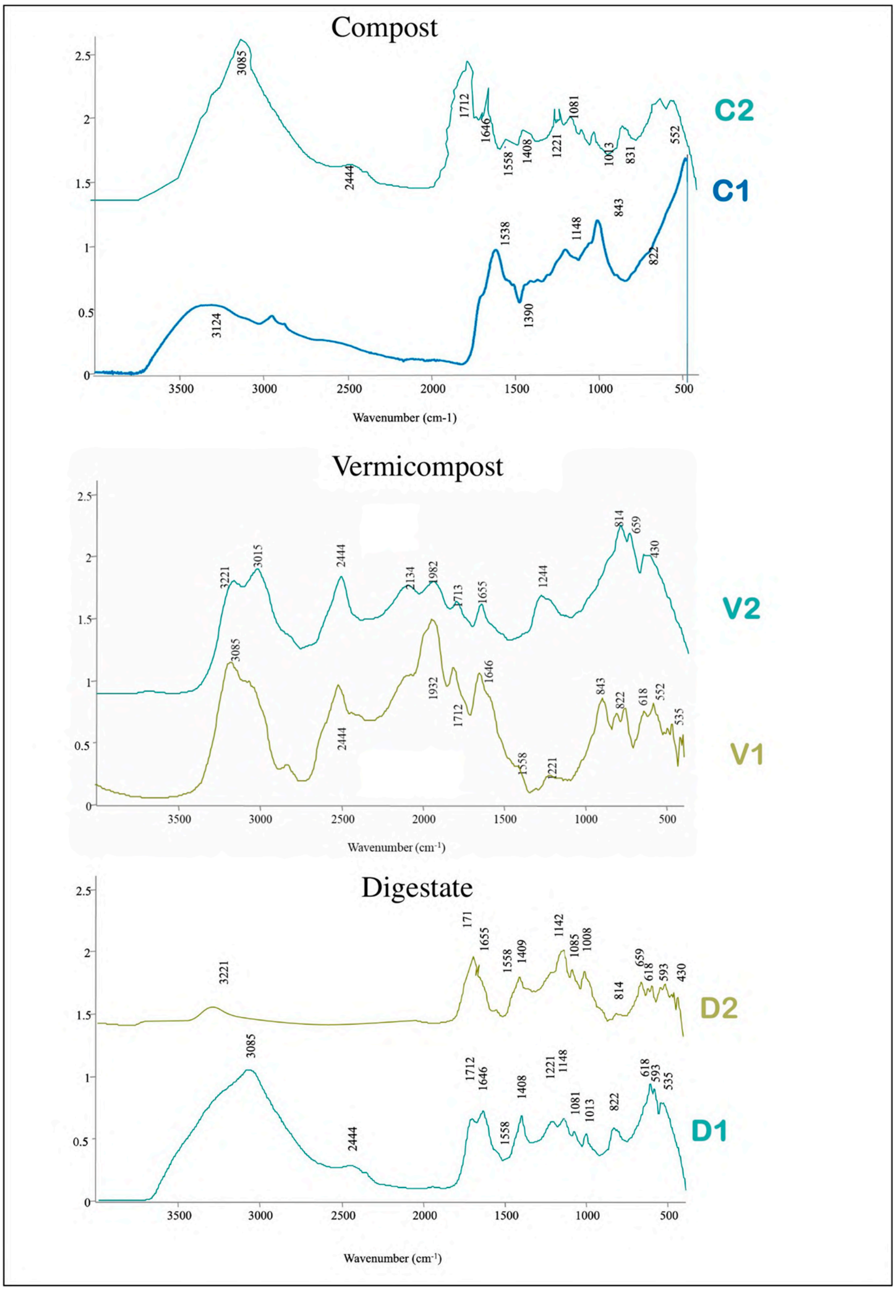

Figure 6. DRIFT spectra of compost C1, C2, D1, D2, V1 and V2.

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.

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Dottorati PON - Bando 2021 - Ciclo 37 (XXXVII)

Azione IV.4 - Dottorati e contratti di ricerca su tematiche dell'innovazione

Azione IV.5 - Dottorati su tematiche Green

UNIONE EUROPEA
FSE REACT-EU

Dottorando	Angela Maffia
Tutor	Adele Maria Muscolo
Co-tutor	Giuseppe Celano
Coordinatore	Leonardo Schena
Corso di Dottorato	Scienze agrarie, alimentari e forestali
Ciclo	XXXVII
Codice borsa e n.	DOT1647787, n.5
CUP	C35F21001320002
Tipologia Green/Innovazione	Green
Titolo Progetto	Production of environmentally sustainable fertilizers from agro-industrial wastes for the rehabilitation of degraded areas and biodiversity protection