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ORIGINAL PAPER

Plant Growth and Root Morphology Are Affected by Earthworm-Driven (*Eisenia* sp.) Changes in Soil Chemico-Physical Properties: a Mesocosm Experiment with Broccoli and Faba Bean

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Abstract
Earthworms are "ecosystem engineers" that improve soil water and nutrient content, soil macroporosity, and aeration, and provide suitable habitats for microbial populations. This study aimed at defining if the presence of epigeic earthworms (*Eisenia* sp.) affected the growth and development of two plant species (*Brassica oleracea* and *Vicia faba*) via the modifications of soil chemico-physical properties. A mesocosm experiment, in which plants were grown outdoors for 4 months with or without earthworms, was performed. The two plant species were selected based on their different habitus and root architecture and morphology. Soil macroporosity (M_{soil}) and water holding capacity (WHC_{soil}) were determined. Earthworm-driven bioturbation (B_{soil}) was measured by filling mesh bags with artificial soil. Earthworm abundance and biomass, together with plant morphometric parameters (root and leaf morphology by imaging and microscope techniques), were measured at the end of the trial. The presence of earthworms increased M_{soil} (on average +16%) and WHC_{soil} (on average +9%) and this was accompanied by a remarkable degree of B_{soil}. In most of the cases, earthworms enhanced plant growth in the two plant species studied, with a significant positive influence on the majority of the shoot and root traits. A significant increase of stomatal density (on average +24%) occurred in the leaves of both the plant species in the presence of earthworms. Our results confirmed the hypothesis that bioturbation by *Eisenia* sp. had a significant positive effect on plant growth, independently from the plant species cultivated, and that these growth-promoting effects were mediated by changes in soil chemico-physical parameters. By taking into account the essential role of earthworms in maintaining healthy soils and the vegetation they support, soils can become more resilient against environmental perturbations and climate change.

Keywords Bioturbation · Earthworms · Plant growth · Roots · Soil fauna · Soil chemico-physical properties

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Abbreviations

BR_{ctrl}	Control broccoli without earthworms
BR_{earth}	Broccoli with earthworms
B_{soil}	Soil bioturbation
CR	Coarse roots
d_{stoma}	Stomatal density
Dw_{root}	Root dry weight
FB_{ctrl}	Control faba bean without earthworms
FB_{earth}	Faba bean with earthworms
FR	Fine roots
F_{root}	Root fineness
Fw_{root}	Root fresh weight
h_{shoot}	Shoot height
l_{CR}	Total root length of coarse roots
l_{FR}	Total root length of fine roots
l_{VFR}	Total root length of very fine roots
Ml_{root}	Root maximum length
MR_{root}	Root mass ratio

M_{soil}	Soil macroporosity
n_{earth}	Earthworm number
n_{fruit}	Fruit number
n_{leaf}	Leaf number
n_{node}	Number of branching nodes
Sa_{leaf}	Single leaf area
Sa_{root}	Root surface area
SD	Standard deviation
SOC	Soil organic carbon
STN	Soil total nitrogen
Ta_{leaf}	Total leaf area
Td_{root}	Root tissue density
Tl_{root}	Total root length
Tw_{earth}	Earthworm total weight
Tw_{fruit}	Fruit total weight
VFR	Very fine roots
V_{root}	Root volume
WHC_{soil}	Soil water holding capacity
w_{leaf}	Leaf weight
w_{stem}	Stem weight
ϕ	Diameter
ϕ_{stem}	Stem diameter

1 Introduction

In agricultural soils, earthworms play a key role in maintaining soil structure, improving water drainage, and enhancing nutrient cycling (Frouz et al. 2015; Bünemann et al. 2018). The physical and chemical changes in the surrounding environment create habitats for other organisms, which leads to consider earthworms as "ecosystem engineers" (Briones 2014; Sofo et al. 2020a). In particular, they significantly increase soil microbial biomass and microbial respiration (Bünemann et al. 2018; Lavelle et al. 2020).

Earthworms can increase crop yield by 25% and aboveground biomass by 23% predominantly through releasing the nitrogen present in residue and soil organic matter (Van Groenigen et al. 2014). Similarly, in another meta-analysis study, Xiao et al. (2018) found that earthworm presence increases plant growth by 20% accompanied by enhanced nitrogen content by 11%. In contrast, other studies did not detect a causal relationship between earthworm abundance, soil fertility, and plant growth promotion (Logsdon and Linden 1992; Hodson et al. 2021).

Bioturbation is defined as the biological reworking of soils or sediments by organisms, including microbes, plant roots, and burrowing animals (Piron et al. 2017; Yu et al. 2017). This process is considered a major driver of ecosystem functioning because it often alters soil physico-chemical properties and biotic interactions, leading to improved soil health and fertility (Richards et al. 2011). Earthworm bioturbation is particularly recognized for its influence on ecosystem services, e.g., nutrient cycling, water flow, control of soil erosion and runoff, and climate regulation (Breuning-Madsen et al. 2017; Tuma et al. 2019).

While field studies often show positive plant responses to high earthworm abundance, microcosm experiments can reveal the mechanisms responsible for earthworm effects on above- and belowground plant growth responses. For root biomass, some studies reported a decrease in the biomass of the fine roots when earthworms are present, although increases in total root length have been also reported (Agapit et al. 2017; Nahberger et al. 2021). These contradictory findings could be the result of the different complexity and multi-dimensionality of the root system, and their different responses to abiotic and biotic stresses, such as nutrient deficiency (Sorgonà and Cacco 2002), drought and extreme temperature (Vescio et al. 2021), and allelopathy (Abenavoli et al. 2016). In addition, root traits, such as root length, root mass, fineness, and tissue density, modulate the responses to environmental change (Ryser 1998). Considering the interactions between roots and earthworm in determining the soil physico-chemical environment, the dynamics of root morphological responses to earthworms needs to be more thoroughly explored.

In this study, we investigated if epigeic earthworms (*Eisenia* sp.) can affect the growth and development of two plant species (*Brassica oleracea* and *Vicia faba*) via the modifications of soil chemico-physical properties. The earthworm chosen is an active epigeic earthworm or surface-dwelling, ubiquitous with a worldwide distribution, and commonly used as a test species for soil quality improvement (Tomati and Galli 1995; Žaltauskaitė et al. 2022). In addition, some authors (Mkhinini et al. 2020) have shown that *Eisenia andrei* can stimulate plant growth and protection against oxidative stress in bean plants (*Vicia faba*) irrigated with treated wastewater. The two plant species were selected based on their different rooting strategies: thin, deep, and dense taproot in broccoli; fibrous, shallow, and diffuse root system in faba bean (Li et al. 2014; Sofo et al. 2020b).

The hypothesis of our study is understanding if *Eisenia* sp. can modify soil macroporosity, water holding capacity, and carbon/nitrogen dynamics by earthworm-driven bioturbation, and if these soil chemico-physical changes can affect plant morphometric parameters and plant growth. The novelty of this experiment, conducted under controlled conditions, resides in the fact that we considered a wide range of soil and plant (root, stem, fruits, leaves) parameters, with an emphasis on the root system, a plant organ often overlooked. Finally, a particular attention was given to the interactions between bioturbation-mediated soil porosity and water content that can influence plant growth and morphology.

2 Materials and Methods

2.1 Experimental Set-up

The experimental area (Trani, BT, Puglia Region, Italy; 41°16′25″32 N, 16°24′58″32 E) is characterized by a semi-arid climate, with an average annual rainfall of 595 mm (1995–2021) and a mean annual temperature of 16.0 °C. The trial was carried out outdoors in the Autumn–Spring 2020–2021 (November–March) using potted plants growing on the same soil and under the identical climatic conditions in a rainfed regime; this allowed the elimination of any indirect effects due to soil type and climate regime.

On 28 November 2020, 2-week-old seedlings of broccoli (*Brassica oleracea* L.) and faba bean (*Vicia faba* L.) were transferred to 90-L conical pots (upper diameter = 60 cm, lower diameter = 35 cm, height = 50 cm) filled with local topsoil (0–30 cm) collected from an adjacent olive orchard: sandy clay with 45% sand, 14% silt, and 41% clay, pH of 7.40 ± 0.03 SD, soil organic carbon 43.3 ± 0.21 SD kg^{-1}, and soil total nitrogen of 1.45 ± 0.02 SD g kg^{-1}, determined according to Pansu and Gautheyrou (2006). Each pot contained one seedling.

Pots were incubated outside. Soil water content measured gravimetrically was not significantly different at the beginning of the experiment. Inoculated and non-inoculated pots were analyzed in four replicates for each plant species. In the eight inoculated pots, approximately 15 g of mature, clitellated earthworms (*Eisenia* sp.) purchased from a local supplier (Fattoria Gallorosso Ssa; Matera, Italy) were added the same day the seedlings were transferred, whereas the eight non-inoculated (control) pots did not contain earthworms. This level of earthworm density here used was comparable to the total earthworm density found in similar agricultural areas around the experimental site, with earthworms living in the same soil and litter type (Sofo et al. 2020c). Fences (height = 15 cm) were placed around mesocosms' tops to prevent earthworms from escaping. During the experimental period, no supplementary food was provided to earthworms.

Therefore, the experimental set-up resulted in four treatments: broccoli with earthworms (BR_{earth}), faba bean with earthworms (FB_{earth}), broccoli without earthworms (BR_{ctrl}), and faba bean without earthworms (FB_{ctrl}). The incubation experiment ended on 31 March 2021.

2.2 Soil Chemico-Physical Analyses

Soil macroporosity (M_{soil}) and soil water holding capacity (WHC_{soil}) were measured at the beginning of the trial (28 November) and at the end (31 March) in soil samples (about 25 and 10 g fresh soil for M_{soil} and WHC_{soil}, respectively) collected at a depth of 10 cm. M_{soil} was determined using a transmitted light microscopy (model ADL 601 P 40-600x; Bresser GmbH, Rhede, Germany) connected to a digital camera (model MikroCam II 20 MP 1″; Bresser GmbH, Rhede, Germany). Soil thin sections (30 μm) embedded in epoxy resin (Durcupan™; Sigma-Aldrich, St. Louis, MI, USA) (Supplementary Fig. S1) were prepared following the method of Sotta and Fujiwara (2017) and macropore total volume was evaluated by Image J software (https://imagej.nih.gov/ij/index.html). Three soil sections per replicate pot (twelve per treatment) were obtained and the values of macroporosity averaged.

WHC_{soil} was estimated at the beginning of the trial on three random soil samples taken from each pot as the weight difference between the soils at field capacity (i.e., samples saturated with water and then weighed after full drainage) and that of the dried soils (oven-dried at 105 °C for 24 h), and expressing the average values as percentages (w/w) (Pansu and Gautheyrou 2006).

Another aliquot (50 g) of the sampled soil was dried at 105 °C for 24 h, placed in a desiccator until they reached a constant weight, and then sieved through a 2-mm stainless steel sieve. Soil organic carbon (*SOC*) was determined by the Walkley and Black method by oxidation at 170 °C with potassium dichromate ($K_2Cr_2O_7$) in the presence of sulfuric acid (H_2SO_4), and the excess $K_2Cr_2O_7$ was measured by Möhr salt titration (Pansu and Gautheyrou 2006). Soil total nitrogen (*STN*) was measured by the Kjeldahl method (Pansu and Gautheyrou 2006).

2.3 Earthworm Abundance and Bioturbation Activities

Earthworms were counted and weighted (earthworm number, n_{earth}, and earthworm total weight, Tw_{earth}, respectively) before their addition to the pots and at the end of the incubation (Fig. 1; Supplementary Fig. S2a).

Two different soil bags (5 cm in diameter, 20 cm high) were prepared to allow/prevent earthworm access; (i) mesh size of 1 mm that allowed microorganisms and small fauna to enter the bags but excluded earthworms; (ii) mesh of 1 mm with additional 10 mm diameter holes located at 5-cm intervals along the bags for allowing earthworm access. On 28 November 2020, for each pot of BR_{earth} and FB_{earth}, two pits (6 cm diameter, 20 cm deep; 35 cm apart from each other) were arranged in pairs in each pot to allocate the two types of soil bags, one with and one without holes. Each mesh bag was filled with 3:1 (w/w) sand/clay mixture using a funnel. The sand (Ø = 0.6–1.2 mm; no. GERB5464; VWR International) and bole white (kaolin clay) (n. MFCD00062311; VWR International, Dublin, Ireland) were oven-dried at 105 °C overnight before use. Finally, the bags were closed using

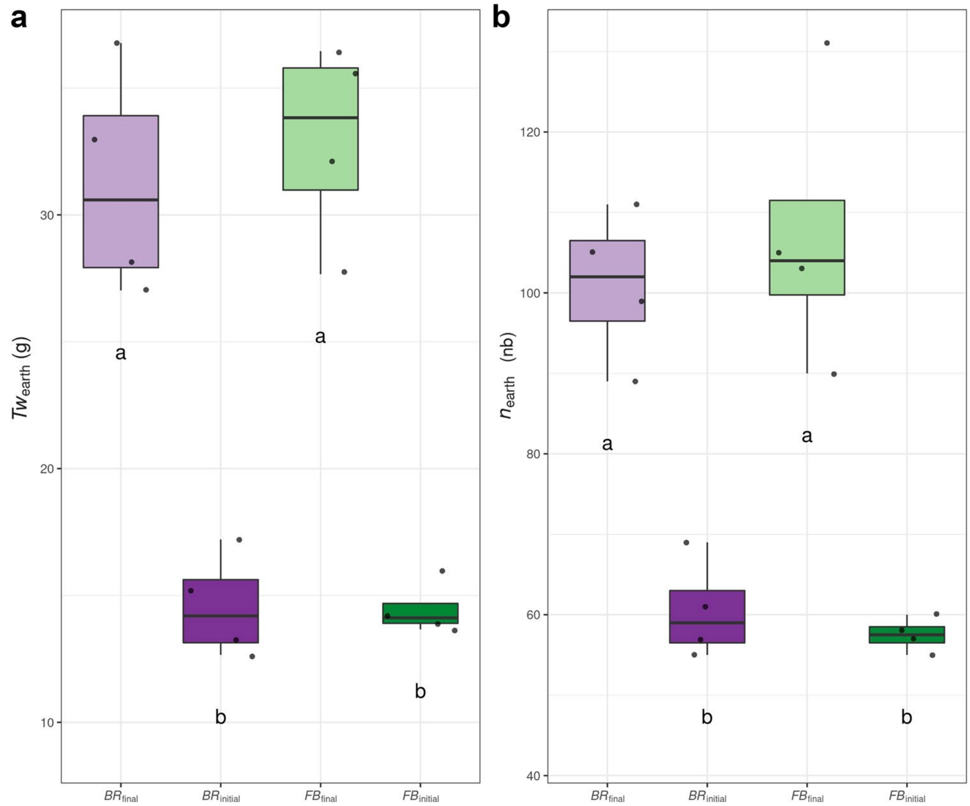

Fig. 1 Initial and final **a** earthworm total weight (Tw_{earth}) and **b** earthworm number (n_{earth}) in the broccoli (*BR*) and faba bean (*FB*) treatments. Significant differences ($p < 0.05$) between treatments are indicated by different letters ($n = 4$, mean ± standard deviation)

a wire. Then, approximately 100 mL of tap water was poured on the surface of the core. Finally, the bags were covered with the excavated soil.

The final destructive sampling occurred on 31 March 2021. The bags were carefully extracted and placed on a tray and cleaned, and the growing roots cut using a knife (Supplementary Fig. S2b). The bags were then opened vertically, and the content subdivided into 0–5, 5–10, and 10–20 cm layers, and air-dried. For each layer, the biogenic structures were easily identified, as they were constituted by aggregates and soil including any organic traces, often having a cylindrical and rounded shape (Supplementary Fig. S2c). The biogenic structures were separated from the pure sand/kaolin mixture by hand and placed in a desiccator until mass constancy for recording their dry mass. The contribution of earthworms alone to soil bioturbation (B_{soil}) was evaluated by subtracting, for each layer, the weight of the biogenic structures collected from the mesh bags with holes (with earthworm access) from the respective values from the mesh bags without holes (no earthworm access). A conceptual scheme of bioturbation measurement is shown in Supplementary Fig. S2d.

2.4 Plant Morphometric Parameters

Plants were carefully removed from the pots after wetting the soil to avoid any root damage. Shoots were separated from the roots and shoot height (h_{shoot}) was measured using a ruler (Supplementary Fig. S3). In the case of the broccoli plants, leaves were abscised at the level of the petiole base using a scalpel. Then, the leaves were counted (leaf number, n_{leaf}), individually weighted (leaf weight, w_{leaf}), and digitally scanned (Canon CanoScan D646U; Canon Electronics Inc., Tokyo, Japan) at a resolution of 600 dpi (Supplementary Fig. S4) for measuring the leaf area (Sa_{leaf}) and the total leaf area (Ta_{leaf}) by ImageJ software. The stems were also individually weighted (stem weight, w_{stem}), and their diameter (ϕ_{stem}) measured at three levels (upper, central, lower) and their mean was calculated. For faba bean plants, four measures were taken: (i) the number and total weight of the beans (n_{fruit} and Tw_{fruit}, respectively), (ii) Ta_{leaf}, calculated on 50 leaves randomly chosen and normalized based on the total number of leaves (n_{leaf}) due to the much higher valued compared to broccoli, (iii) the number of branching nodes (n_{node}) (no branching is present in broccoli), and (iv) the diameter of the longest stem, at six points and not three as in broccoli.

Stomatal density (d_{stoma}) was evaluated with an incident/transmitted light microscopy (model ADL 601 P 40-600x; Bresser GmbH, Rhede, Germany) connected to a digital camera (model MikroCam II 20 MP 1″; Bresser GmbH, Rhede, Germany) (Supplementary Fig. S4). Three random leaves were taken from the upper part of the shoot and each plant. The lower surface of each leaf was coated with transparent varnish and let dry. Then, the dried area was peeled off by using transparent sellotape, and the leaf epidermal layer was placed on a microscope slide. Ten areas without visible leaf veins were randomly chosen for measuring d_{stoma}, which was then expressed as cm^2 of the total leaf surface.

The plant roots were cleaned by washing off the excess of soil using tap water. The maximum root length (Ml_{root}), which is an estimate of the rooting depth, was measured using a ruler and then, all roots were preserved in an 80:20 (v/v) water:ethanol solution. The root morphological analysis was carried out using a root-specific image analysis system (WinRhizo; Instruments Régent Inc., Chemin Sainte-Foy, Québec, Canada). Previously, the roots were stained with 0.1% toluidine blue solution for 5 min and then scanned at a resolution of 600 dpi (WinRhizo STD 1600; Instruments Régent Inc.). The WinRhizo Pro v. 4.0 software package (Instruments Régent Inc.; Chemin Sainte-Foy, Québec, Canada) was used to measure the total root length (Tl_{root}), root surface area (Sa_{root}), and root volume (V_{root}). In addition, the root length distribution according to the diameter classes defined by Bohm (1979) was estimated: coarse (l_{CR}, > 1.0 mm), fine (l_{FR}, 0.5–1.0 mm), and very fine root length (l_{VFR}, 0–0.5 mm). Thereafter, the fresh weight (Fw_{root}) and dry weight of the roots (after oven-drying at 70 °C for 48 h, Dw_{root}) were measured. Finally, three root length parameters were also calculated: (i) root mass ratio (MR_{root}, root dry weight/whole plant dry weight), (ii) root fineness (F_{root}, root length/root volume), and (ii) root tissue density (Td_{root}, root dry weight/root volume).

2.5 Statistical Analyses

Statistical differences between treatments were assessed by means of ANOVA using the R package (https://www.r-project.org/), with plant species (broccoli and faba bean) and earthworm treatments (earthworms and control) as fixed factors. All data were firstly checked for normality (Kolmogorov–Smirnoff test) and homogeneity of variance (Levene median test). Tukey's test was used as a post hoc test ($p < 0.05$).

3 Results

3.1 Earthworm Effects on Soil Properties and Bioturbation

The number and biomass of earthworms (Tw_{earth} and n_{earth}) significantly increased in the two plant treatments (BR_{earth} and FB_{earth}) over the course of the experiment, with the highest values being observed in the FB_{earth} treatment (32.94 g and 107 specimens, respectively) at the end of the experimental period (Fig. 1).

The increased abundance of earthworms significantly affected M_{soil}, which increased by +15.4 and +17.9% in BR_{earth} and FB_{earth}, compared to initial values (10.66% ± 0.52 SD; n = 16) (Fig. 2a). In the case of those treatments without earthworms (BR_{ctrl} and FB_{ctrl}), the increases in M_{soil} were less marked (+8.2 and +4.1%, respectively) (Fig. 2a). At the end of the trial, there was an increase in WHC_{soil} at both BR_{earth} and FB_{earth} treatments by 9.0 and 8.6%, respectively, when compared to the initial values (64.9 g ± 2.5 SD; n = 16) (Fig. 2b). In the case of the treatments without earthworms, the increases in *WHC* were less noticeable (4.0 and 3.1% in BR_{ctrl} and FB_{ctrl}, respectively) (Fig. 2b). The values of *SOC* decreased in both the treatments with earthworms (BR_{earth} and FB_{earth}) compared to the initial conditions (Table 1). Independently from the earthworms, no significant reductions in *STN* were observed in faba bean soils, while the values in broccoli soils were significantly lower compared to those at the beginning of the experiment (Table 1).

The weight of the biogenic structures in the soil bags without earthworm access was negligible across all treatments and for all the three soil depths (on average, 0.16, 0.19, and 0.31 g at soil depths of 0–5, 5–10, and 10–20 cm, respectively; n = 16). In contrast, a significant increase in the weight of biogenic structures was observed in the mesh bags with earthworm access, for the two plant treatments (BR_{earth} and FB_{earth}). Furthermore, within each plant treatment, significant differences were observed in the abundance of biogenic structures at the 10–20 cm soil layer (10.19 vs 0.28 g in BR_{earth} and BR_{ctrl}, respectively, and 8.16 vs 0.30 g in FB_{earth} and FB_{ctrl}, respectively; n = 4). At 5–10 and 10–20 cm soil layers, the weight of the biogenic structures was significantly higher in BR_{earth} than in FB_{earth} (Fig. 3b, c).

3.2 Earthworm Effects on Plant Traits

Plant growth was significantly enhanced in the presence of earthworms (Figs. 4, 5, and 6). In the case of faba bean, this was evidenced by the significantly higher values of shoot height (h_{shoot}) when earthworms were present (63.4 vs 51.0 cm in FB_{earth} and FB_{ctrl}, respectively) and stem weight (w_{stem}) (177.12 vs 145.55 g in FB_{earth} and FB_{ctrl}, respectively) (Fig. 4a, c). Regarding the leaf area, similar earthworm-driven effects were observed for Ta_{leaf} in both plant treatments (2757.43 vs 2146.25 g in BR_{earth} and BR_{ctrl}, respectively; and 2430.09 vs 1952.83 g in FB_{earth} and FB_{ctrl}, respectively). However, Sa_{leaf} was significantly affected by earthworm presence in the broccoli treatment only (297.10 vs 247.07 g in BR_{earth} and BR_{ctrl}, respectively) (Fig. 4e, f). A significant increase in d_{stoma} was observed in the leaves of both plant species in response to earthworms (Fig. 4g). Significant increases in n_{fruit}, Tw_{fruit}, and n_{leaf} were also measured in faba bean when earthworms were present (Fig. 5a–c).

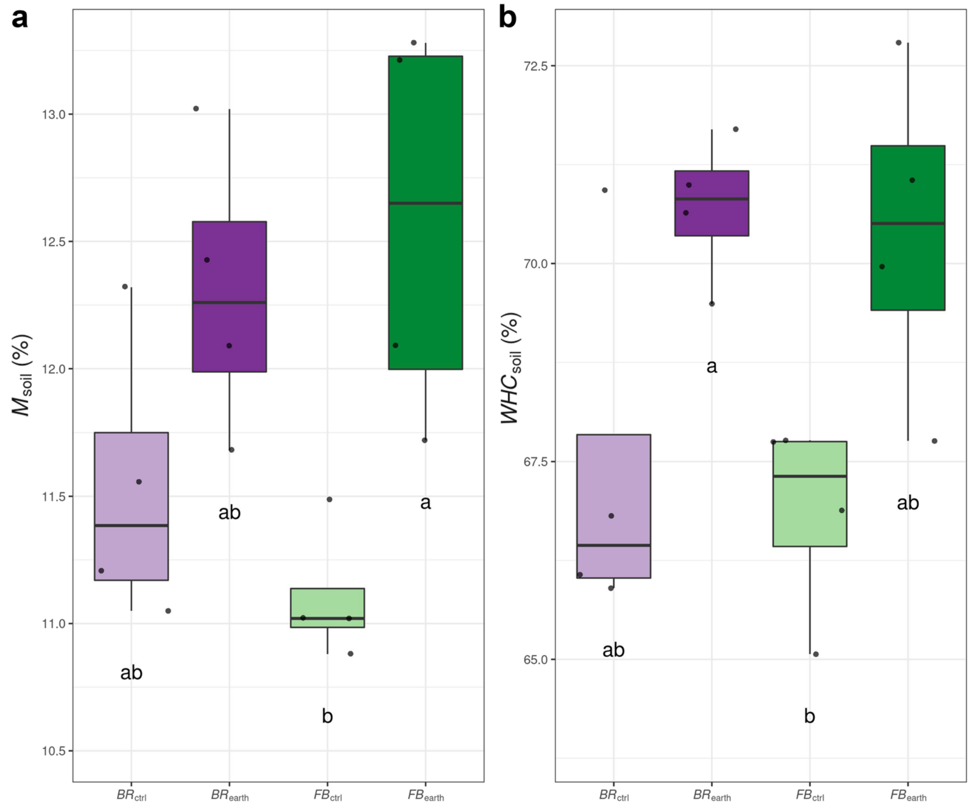

Fig. 2 Boxplots of **a** soil macroporosity (M_{soil}) and **b** soil water holding capacity (WHC_{soil}) in the different treatments, consisting of broccoli without earthworms (BR_{ctrl}), broccoli with earthworms (BR_{earth}), faba bean without earthworms (FB_{ctrl}), and faba bean with earthworms (FB_{earth}). Significant differences ($p <$ 0.05) between treatments are indicated by different letters (n = 4, mean ± standard deviation)

The presence of earthworms also had a significant influence on the majority of the root traits measured except for l_{FR} ($p = 0.06$), l_{CR} ($p = 0.41$), MR_{root} ($p = 0.15$), and F_{root} ($p = 0.59$) (Fig. 6h–k). However, this earthworm effect differed between plant species as evidenced by the significant treatment × species interaction. More specifically, roots were deeper (Ml_{root}: 69.0 vs 55.2 cm in BR_{earth} and BR_{ctrl}, respectively) and better developed (Dw_{root}: 76.82 vs 63.09 g in BR_{earth} and BR_{ctrl}, respectively) in the broccoli treatment with earthworms. Similar effects were not observed in the faba bean treatments (Ml_{root}: 37.2 vs 33.3 cm in FB_{earth} and FB_{ctrl}, respectively; Dw_{root}: 19.27 vs 16.52 g in FB_{earth} and FB_{ctrl}, respectively) (Fig. 6a, c). In contrast, Fw_{root} was not statistically affected by earthworm presence in any of the two plant treatments (317.06 vs 270.08 g in BR_{earth} and BR_{ctrl}, respectively, and 192.00 vs 157.78 g in FB_{earth} and FB_{ctrl}, respectively) (Fig. 6b).

The root systems were well developed, in terms of Tl_{root} and SA_{root}, in the presence of earthworms, but differed between plant treatments, as evidenced by the significant treatment × species interaction ($p = 0.0055$ and 0.0005 for root length and surface, respectively). Accordingly, Tl_{root} and Sa_{root} of broccoli were higher in the presence of earthworms: 458.25 vs 320.30 cm of Tl_{root} in BR_{earth} and BR_{ctrl}, respectively, and 44.64 vs 31.79 cm^2 of Sa_{root} in BR_{earth} and BR_{ctrl}, respectively (Fig. 6d, e).

The root biomass parameters (MR_{root}, and the F_{root}) were not influenced by either the plant or earthworm

Table 1 Soil organic carbon (SOC) and soil total nitrogen (STN) measured at the beginning (28 November) and the end (31 March) of the trial in the different treatments, consisting of broccoli without earthworms (BR_{ctrl}), broccoli with earthworms (BR_{earth}), faba bean without earthworms (FB_{ctrl}), and faba bean with earthworms (FB_{earth}). Significant differences ($p < 0.05$) between treatments are indicated by different letters (lowercase between columns and uppercase between rows) ($n = 4$, mean ± standard deviation)

Treatment	Beginning		End	
	SOC (g kg^{-1})	STN (g kg^{-1})	SOC (g kg^{-1})	STN (g kg^{-1})
BR_{ctrl}	43.1 ± 0.19 aA	1.45 ± 0.05 aA	42.8 ± 1.18 aA	1.28 ± 0.05 bB
BR_{earth}	43.5 ± 0.35 aA	1.47 ± 0.07 aA	39.7 ± 0.25 bB	1.21 ± 0.04 cB
FB_{ctrl}	43.0 ± 0.39 aA	1.39 ± 0.10 aA	42.5 ± 0.91 aA	1.45 ± 0.03 aA
FB_{earth}	42.9 ± 0.50 aA	1.48 ± 0.09 aA	40.5 ± 0.16 bB	1.33 ± 0.04 bA

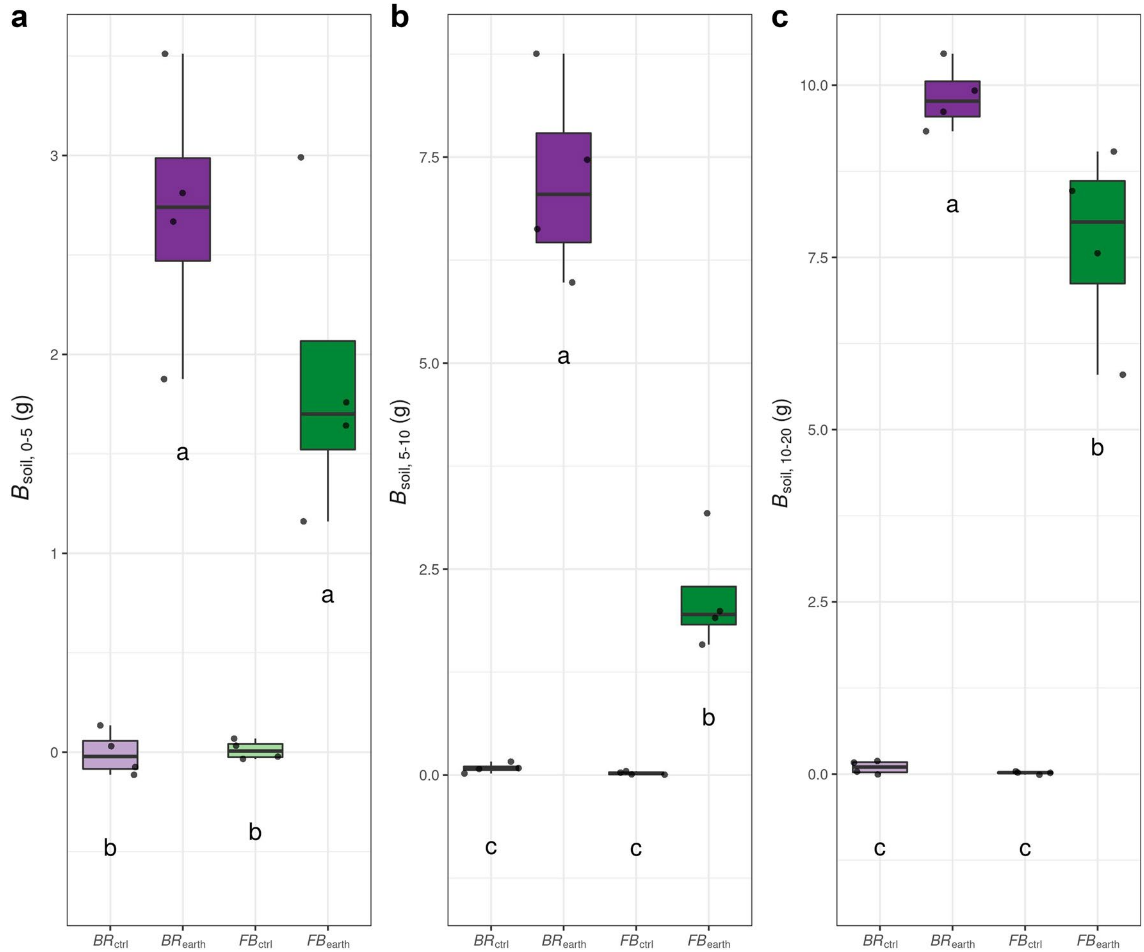

Fig. 3 Boxplots of soil bioturbation (B_{soil}) at the soil depths of **a** 0–5 cm, **b** 5–10 cm, and **c** 10–20 cm in the different treatments, consisting of broccoli without earthworms (BR_{ctrl}), broccoli with earthworms (BR_{earth}), faba bean without earthworms (FB_{ctrl}), and faba bean with earthworms (FB_{earth}). Significant differences (p < 0.05) between treatments are indicated by different letters (n = 4, mean ± standard deviation)

treatments, nor by the treatment × species interaction (Fig. 6a, k). Conversely, the Td_{root} was significantly higher in control treatments than in the presence of earthworms: 0.123 vs 0.105 g cm^{-3}, respectively (Fig. 6l). In addition, as observed for Tl_{root} and Sa_{root}, the effect of earthworms on Td_{root} was also affected by plant species (significant treatment × species interaction, $p = 0.0013$) and thus, significant differences were only observed for broccoli (0.19 vs 0.14 g cm^{-3} in BR_{ctrl} and BR_{earth}, respectively; Fig. 6l).

The morphological analysis of the root systems indicated that only the l_{VFR} was significantly modified by earthworms, as an increase of 68% was observed compared to the control treatments (Fig. 6g). As before, the treatment × species interaction was statistically significant (p = 0.0081), and the l_{VFR} of broccoli responded to the earthworm treatments, differently to that of faba bean one (Fig. 6g).

4 Discussion

Earthworm biogenic structures (burrows and casts) are known to have a strong effect on soil porosity, water hydraulic properties, soil carbon dynamics, and microbial populations (Frouz et al. 2015; Piron et al. 2017; Sofo et al. 2020c). While burrows are produced during excavation, casts are the result of digestion processes. In our system, earthworm bioturbation activities (B_{soil}) were accompanied by the physical changes observed in the soil. Furthermore, the influence of earthworms on M_{soil} was likely a major factor responsible for the increases in WHC_{soil} observed in both plant treatments (BR_{earth} and FB_{earth}), as a result of the biomacropores getting filled with water. Notably, earthworms can significantly modify soil water retention, and plant photosynthesis and evaporation rates,

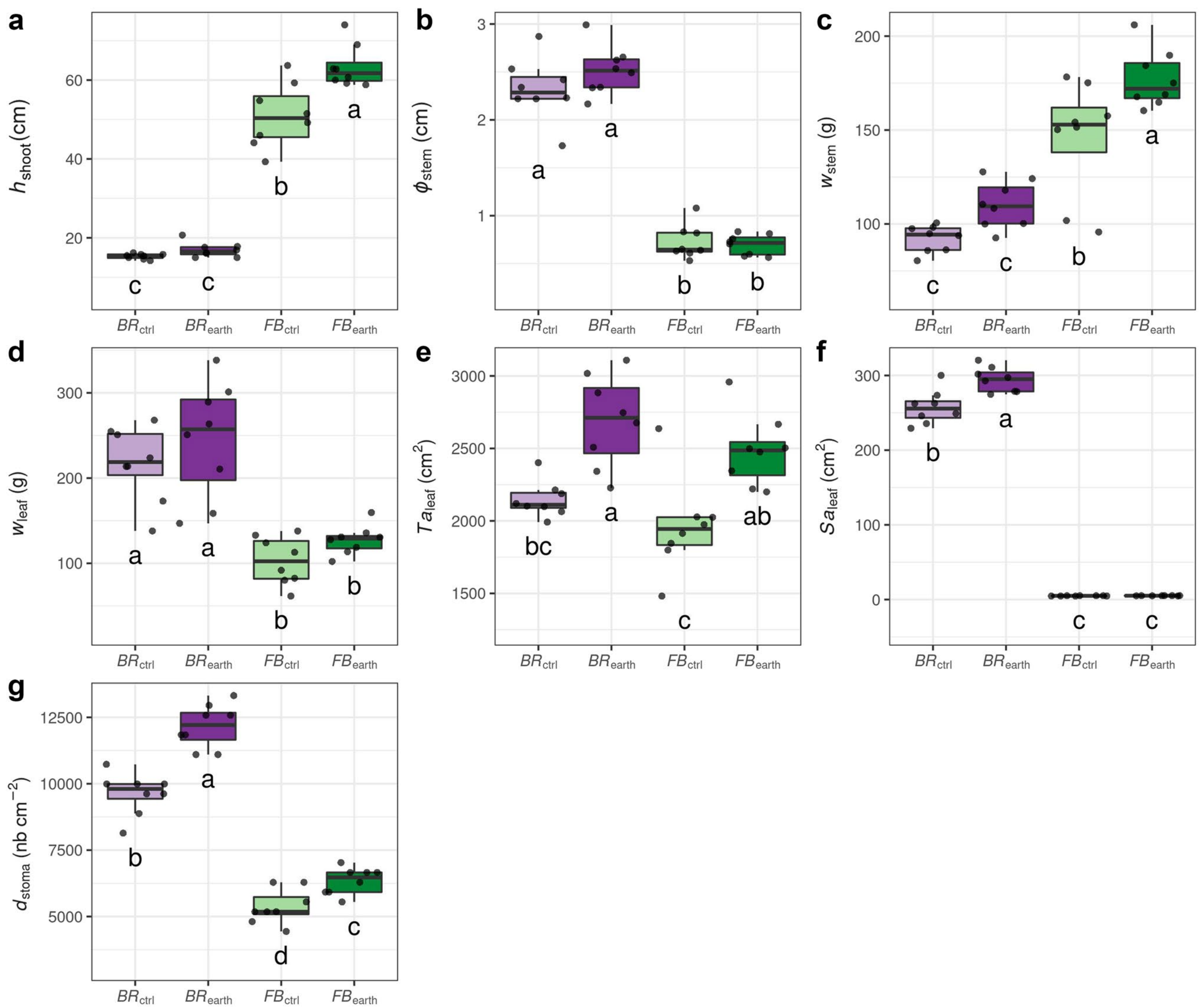

Fig. 4 Boxplots of **a** shoot height (h_{shoot}), **b** stem diameter (ϕ_{stem}), **c** stem weight (w_{stem}), **d** leaf weight (w_{leaf}), **e** total leaf area (Ta_{leaf}), **f** single leaf area (Sa_{leaf}), and **g** stomatal density (d_{stoma}) in the different treatments, consisting of broccoli without earthworms (BR_{ctrl}), broccoli with earthworms (BR_{earth}), faba bean without earthworms (FB_{ctrl}), and faba bean with earthworms (FB_{earth}). Significant differences ($p < 0.05$) between treatments are indicated by different letters ($n = 8$, mean ± standard deviation)

as demonstrated by Blouin et al. (2007) in soils with *Millsonia anomala* maintained at field capacity. On the other hand, the slight increases in soil macroporosity (M_{soil}) and WHC_{soil} measured in the two plant treatments without earthworms (BR_{ctrl} and FB_{ctrl}) can be attributed to roots growing (Van Groenigen et al. 2014; Sofo et al. 2020c).

It is noteworthy that the majority of the earthworm burrows are colonized by roots that benefit from the minimal soil resistance and the greater availability of nutrients, water, and air (Logsdon and Linden 1992; Bhadauria and Saxena 2010; Van Groenigen et al. 2019). Moreover, Yaghoubi Khanghahi et al. (2021) found that plants can exhibit different phenotypic responses and create different soil microhabitats, via adjusted root architecture (Springett and Gray 1997).

A depletion of carbon (*SOC*) was observed in the presence of earthworms, likely due to a higher organic matter mineralization, while soil nitrogen (*STN*) levels, in faba bean, remained high due to nitrogen fixation in root nodules that were observed in the roots of faba bean. Moreover, *STN* values were significantly higher in control soils than in BR_{earth} and FB_{earth}, suggesting that, in the presence of earthworms, the readily available soil N was absorbed by roots or, alternatively, leached from the soil.

Together with the changes in soil chemical and physical properties, the observed increase in plant growth could

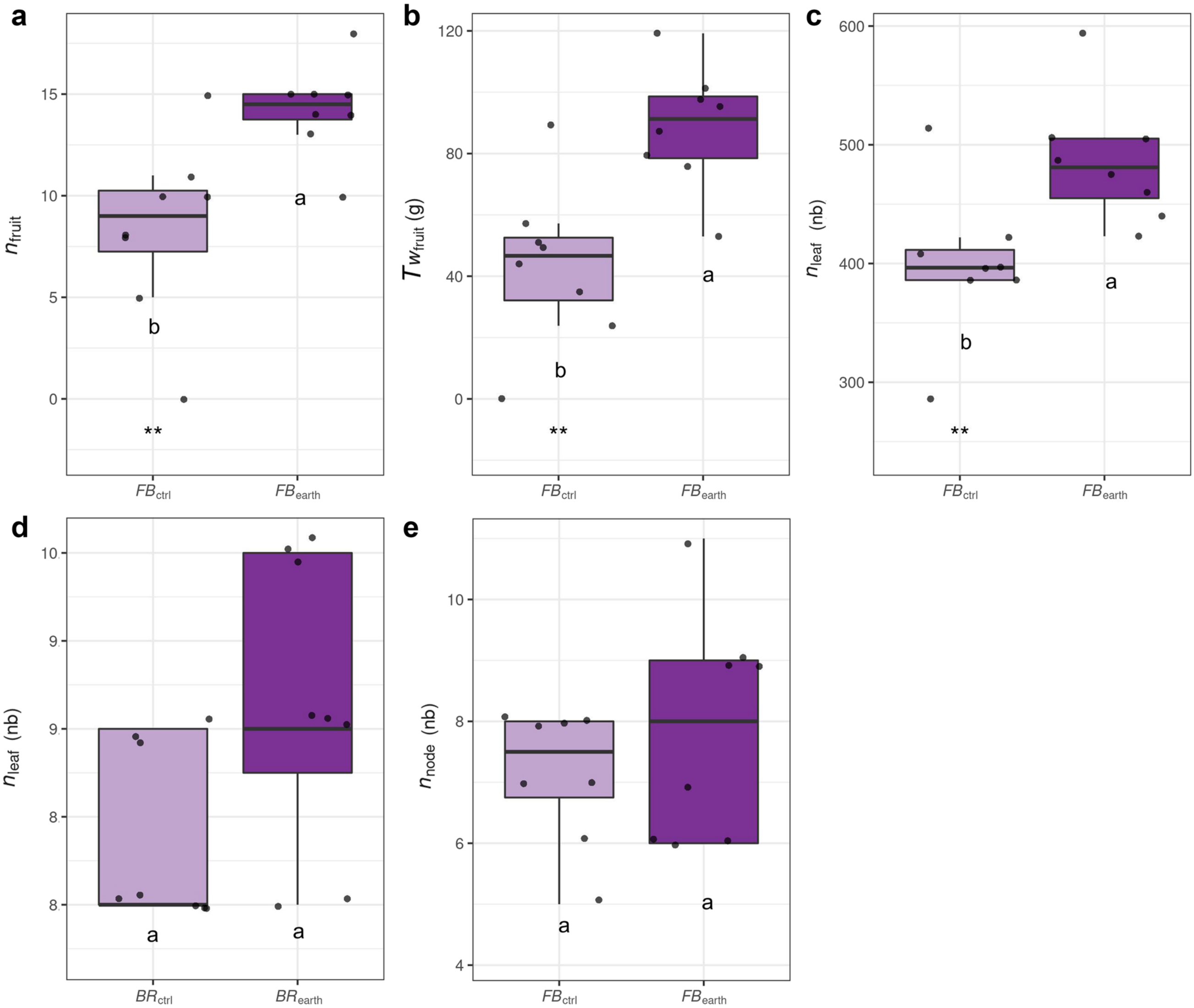

Fig. 5 Boxplots of **a** fruit number (n_{fruit}), **b** fruit total weight (Tw_{fruit}), **c**, **d** leaf number, and **e** number of branching nodes (n_{node}) in the different treatments, consisting of broccoli without earthworms (BR_{ctrl}), broccoli with earthworms (BR_{earth}), faba bean without earthworms (FB_{ctrl}), and faba bean with earthworms (FB_{earth}). Significant differences ($p < 0.05$) between treatments are indicated by different letters ($n = 4$, mean ± standard deviation)

also be attributed to earthworms through the promotion of those soil microorganisms that are beneficial to plants (Scheu 2003; Lavelle et al. 2014) or to changes in the soil microbiota brought about by earthworm activity (Blouin et al. 2007; Hodson et al. 2021). It has also been found that earthworm casts contain hormone-like compounds similar to plant growth regulators, such as gibberellins, cytokinins, and auxins (Tomati et al. 1988; Tomati and Galli 1995). Moreover, Atiyeh et al. (2002) found that the humic acids formed during the breakdown of organic wastes by earthworms can also have a hormone-like role and promote plant growth, in terms of plant height, leaf area, and shoot and root dry weight.

Leaf area architecture is an indicator of general plant physiological status (Rambo et al. 2010; Parkash and Singh 2020), and our results showed that earthworm presence significantly increased the values of d_{stoma} of both plant species, suggesting a better control over water loss and CO_2 uptake. This is the first time this earthworm effect is recorded and provides a strong indication that plants could respond to earthworm activities by adjusting the stomatal density of newly forming leaves to optimize their investment in growth and evapotranspiration.

The root system determines plant capacities for nutrient and water acquisition (Li et al. 2012; Abenavoli et al. 2016; Griffiths et al. 2021), exudation and microbiota recruiting

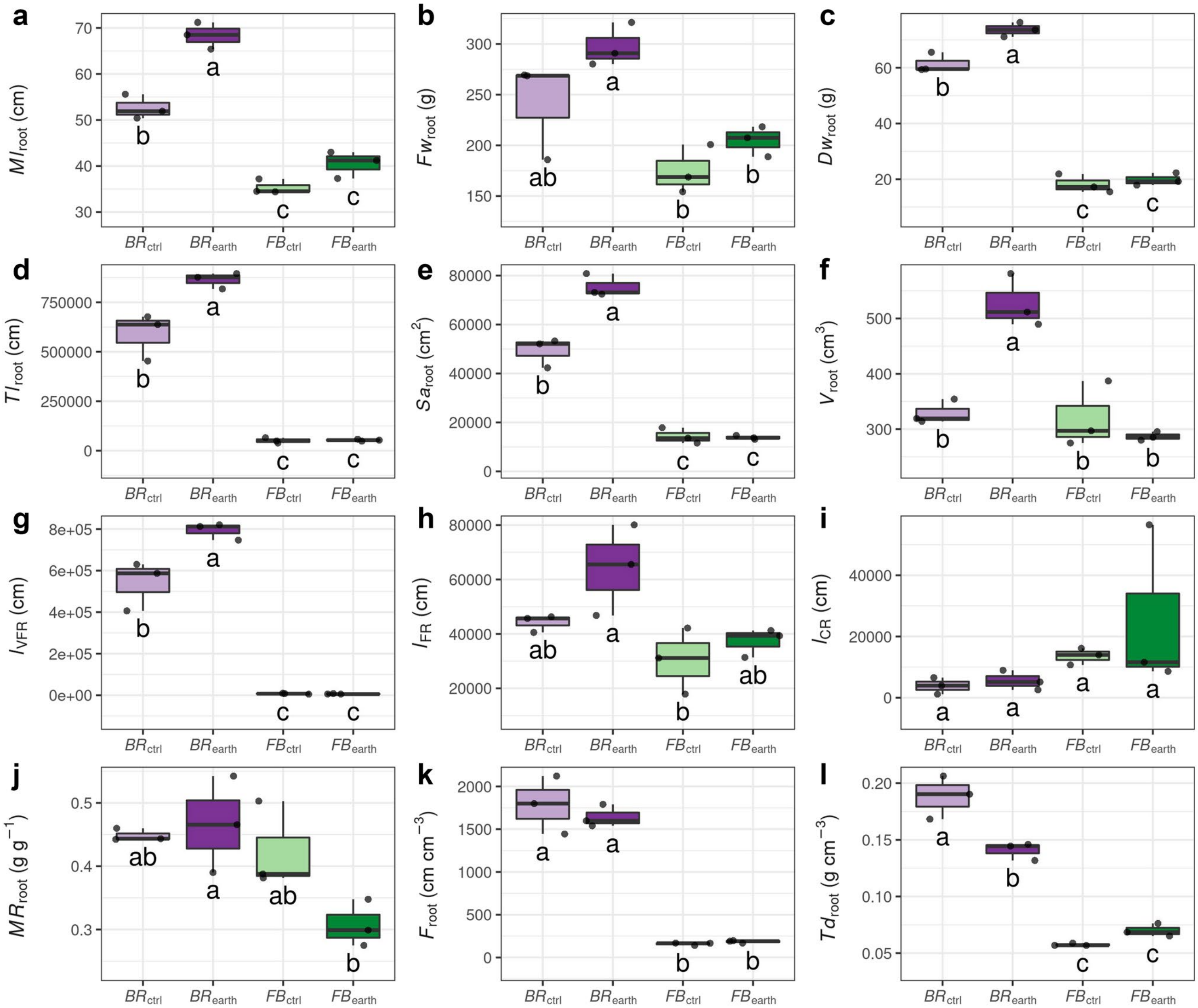

Fig. 6 Boxplots of **a** root maximum length (Ml_{root}), **b** root fresh weight (Fw_{root}), **c** root dry weight (Dw_{root}), **d** total root length (Tl_{root}), **e** root surface area (Sa_{root}), **f** root volume (V_{root}), **g** total root length of very fine roots (l_{VFR}), **h** total root length of fine roots (l_{FR}), **i** total root length of coarse roots (l_{CR}), **j** root mass ratio (MR_{root}), **k** root fineness (F_{root}), and **l** root tissue density (Td_{root}) in the different treatments, consisting of broccoli without earthworms (BR_{ctrl}), broccoli with earthworms (BR_{earth}), faba bean without earthworms (FB_{ctrl}), and faba bean with earthworms (FB_{earth}). Significant differences ($p < 0.05$) between treatments are indicated by different letters ($n = 4$, mean ± standard deviation)

(Vescio et al. 2021), and hence overall plant performance (Lynch 1995) and tolerance to abiotic and biotic stressors (Abenavoli et al. 2016). In this study, earthworms significantly modified the root morphology in terms of root biomass and density as well as root length, in agreement with previous studies (Castellanos Suarez et al. 2014; Agapit et al. 2017, 2018). Therefore, it is possible to suggest that earthworm bioturbation activities result in nutrients and water being more evenly distributed in the soil matrix, and plants respond to these physico-chemical changes by developing longer/deeper and fine roots to increase soil exploration. This greater nutrient and water acquisition by roots leads to higher plant growth and biomass. Therefore, root density/root length ratio can be used as a proxy for estimating the root cost-benefit ratio of plant growth strategies in different soils or under different soil conditions (Ryser 1998; Arnone and Zaller 2014). Accordingly, a lower Td_{root} means a lower number of lengthy roots and, in our study, the presence of earthworms resulted in a lower Td_{root}, suggesting a higher investment in root growth rather than on producing new roots.

These earthworm-induced modifications in the root morphology varied depending on the plant species growing on the soil, with the root system of broccoli being more responsive than that of faba bean to earthworm bioturbation activities, and confirm previous results showing that earthworm effects on plant productivity disappear when legumes are present (Van Groenigen et al. 2014). The root system of the brassica plant produced longer, deeper, and finer roots (i.e., low Td_{root}) when earthworms were present. However, these changes belowground did not correspond with similar changes aboveground. According to a meta-analysis study (Van Groenigen et al. 2014), earthworm density exerts a strong influence on aboveground plant biomass, in particular at high (unrealistic) population numbers of > 400 individuals m^{-2}, whereas at lower densities, the positive effect of earthworms on aboveground biomass varied between +10 and +21%. This could explain why we did not observe a significant increase in aboveground plant productivity at the more realistic earthworm densities used in this study.

5 Conclusions

Taken all together, our results confirmed the hypothesis that soil bioturbation by *Eisenia* sp. had a significant positive effect on plant growth, independently from the plant species cultivated, and that these growth-promoting effects were mediated by changes in soil chemico-physical parameters. The earthworms belonging to *Eisenia* sp. are classified as epigeic species but we demonstrated a certain level of soil bioturbation at a depth of 0–20 cm that, in turn, affected positively soil properties and plant growth. Considering that the topsoil is the most fertile and important root habitat for many herbaceous crops, the findings of our study could be relevant for agricultural purposes. From an applicative point of view, sustainable agricultural management should be designed so that soil fauna activities are enhanced, and earthworms can play a key role by increasing water retention and nutrient cycling while reducing soil erosion. This is particularly urgent and relevant nowadays, as soils are extremely exploited and are subjected to degradation and loss of biodiversity. By taking into account the essential role of earthworms in maintaining healthy soils and the vegetation they support, soils will become more resilient against environmental perturbations and climate change, and will continue to provide the essential environmental, social, and economic benefits that human kind are highly dependent on.

Even if ours was a mesocosm experiment, the reported findings fit into a bigger picture that includes other research fields, such as soil ecology and related ecosystem services, socio-economic advantages of nature-based solutions for sustainable soil management, and potential management/policy applications.

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Author Contribution Conceptualization and experimental set-up were designed by Adriano Sofo. Material preparation, data collection, and analysis were performed by Adriano Sofo, Agostino Sorgonà, and Francesco Reyes. The first draft of the manuscript was written by Adriano Sofo, Maria J.I. Briones, and Agostino Sorgonà and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability Data used in this article will be available on request.

Declarations

Competing Interests The authors declare no competing interests.

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