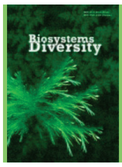

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The interactions between nematode and microbial communities offer significant insights into the impact of organic amendments on the productivity of *Miscanthus* × *giganteus* cultivated on marginal lands

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The investigation is devoted to the analysis of the impact of organic fertilisers, in particular biochar derived from sewage sludge, on the productivity of the energy crop *Miscanthus* × *giganteus* and soil health on marginal lands. The results of long-term observations show that among organic additives, biochar demonstrates the most pronounced stabilising effect on the structure of the nematode community, although its impact on the total number of nematodes is limited. The application of biochar increased the diversity of trophic groups and modified the soil trophic network, which was dose-dependent. The experiment also revealed a significant impact of organic additives on the ecological indicators of nematode communities. In particular, the use of biochar significantly increased the Maturity Index (MI) and the Structural Index (SI), which indicates an improvement in the stability and complexity of the soil ecosystem. Reducing the number of migratory endoparasites and other plant-parasitic nematodes, such as *Pratylenchus* spp. was crucial in increasing the yield of *M.* × *giganteus*. The most pronounced changes were observed when biochar was applied at 10% (BD2). The field trial also assessed the impact of organic amendments on soil microbiological characteristics. Although the total number of bacteria and fungi did not change significantly, there was an increase in the number of *Pseudomonas* bacteria in the biochar-treated samples. This confirms the role of biochar as a stimulator of the growth of beneficial microorganisms and improvement of soil microbial activity. At the same time, the activity of dehydrogenase, which is an indicator of microbial activity, did not change significantly under the influence of additives. The test findings indicate that the addition of biochar has a beneficial effect on the yield of *M.* × *giganteus*, contributing to an increase in green mass at harvest. The impact of organic amendments was long-lasting, demonstrating the potential to increase the productivity of energy crops on marginal lands. Reducing the number of plant-parasitic nematodes, especially migratory endoparasites, was a key factor in improving yields. The outcomes of the study confirm the significance of an integrated approach to the application of organic amendments to improve soil health and increase the productivity of energy crops. The dose-dependent effects of biochar indicate the need to adapt application strategies to specific agroecosystems. Further research should focus on analysing the long-term effects of organic fertilisers on the functioning of trophic networks and microbiological processes in the soil.

Keywords: productivity; soil health; soil biota; trophic groups; plant-parasitic nematodes.

Introduction

In the last two decades, the utilization of marginal or abandoned land has been proposed as a viable long-term solution for the production of energy crops intended for bioenergy processing (Mamirova & Pidlisnyuk, 2024). This approach mitigates competition with productive agricultural land and contributes to the transition toward a bioeconomy (Zymaroieva et al., 2021). In addition to providing renewable biomass feedstocks for energy and high-value biobased commodities, marginal land offers diverse ecosystem services (Kunakh et al., 2022), such as minimizing water and wind erosion (Molozhon et al., 2023), sequestering carbon (Panchenko, 2022; Panchenko et al., 2024), mitigating climate change (Mykhailyuk et al., 2023), improving soil health (Agostini et al., 2015; Dauber & Miyake, 2016), and enhancing biodiversity (Burland & von Cossel, 2023; Kunakh et al., 2023). The economic, agronomic, and environmental feasibility of utilizing biomass is closely linked to maintaining stable yields, which are challenging to achieve due to the low organic carbon content in marginal soils (De Laporte & Ripplinger, 2019). The restoration of marginal land productivity, the cultivation of lignocellulosic biomass feedstock, and improvements in soil health are often initiated through the incorporation of various organic soil amendments (Raniak & Izakovičová, 2024). These materials include agricultural waste, sewage sludge, and derived products such as composted waste, which encompasses municipal solid waste, green waste, food waste, and other non-composted substances. Waste can be utilized directly or transformed into high-value materials, known as biochar (Andrusiv et al., 2024).

Biochar is a carbon-rich substance formed through the pyrolysis of organic waste from various sources. This material is commonly utilized as a soil amendment to enhance crop productivity and improve the biological and physicochemical properties of soil, thereby mitigating the impacts of climate change by increasing carbon storage and reducing greenhouse gas (GHG) emissions (Pidlisnyuk et al., 2021). Furthermore, biochar plays multiple roles in the biological functions of soil by enhancing the activity and biomass of microbial communities. It increases bacterial diversity, which promotes the development of bacterial grazers such as protozoa, nematodes, and enchytraeids (Huang et al., 2023). The effects of biochar on soil biota have been studied for approximately 30 years (Lehmann et al., 2006). However, the existing published data on the interactions between biochar and nematode community structure are limited and often contradictory. Several studies revealed the absence of biochar influence on nematode communities (Pressler et al., 2017; Soong et al., 2017). In contrast, Zhang et al. (2013) reported a significant increase in fungivore abundance alongside a reduction in the abundance of plant-parasitic nematodes. Some researchers, such as Cole et al. (2021) found that the application of 8.0% biochar had no effect on overall nematode abundance; however, it did alter certain nematode populations by decreasing the PPN population and increasing the abundance of predatory nematodes. Conversely, Liu et al. (2020) reported that the addition of 4.5 t/ha of peanut shell-derived biochar enhanced the overall abundance of nematode communities, particularly bacterivores. Several studies have indicated a significant reduction in plant-parasitic nematodes in global cropping systems following the incorporation of biochar produced from various waste sources: 1) agricultural, 2) food processing (Kekelis et al., 2022), and 3) forest processing (Suci Rahayu & Puspita Sari, 2017; Herbrich et al., 2018).

The response of soil nematodes to the application of biochar derived from sewage sludge has not yet been thoroughly evaluated. Our previous research investigated the effects of two biochar doses (BD1 – 5.0% and BD2 – 10.0%) alongside three other organic amendments: sewage sludge (SS), digestate (D), and hemicellulose waste (HW) on the communities of free-living and plant-parasitic nematodes during the first year of *Miscanthus x giganteus* cultivation on marginal land (Stefanovska et al., 2022). The results indicated that the community structure was more mature for SS and less stable for D; however, the impact of biochar on nematode communities remained inconclusive. Consequently, it is essential to continue monitoring the state of the nematode community during the second year of *M.* × *giganteus* growth and to assess the interactions between nematodes and the soil microbial community.

The objectives of this study were as follows: i) to analyze the soil nematode assemblage in amended marginal soil during the second year of treatment with *M.* × *giganteus* vegetation, and ii) to explore the functional significance of nematode-microorganism interactions in the marginal soil affected by the application of biochar and other soil amendments.

Materials and methods

Experimental field. Miscanthus × *giganteus* plantations were established in 2021 on marginal land located in Nové Spoice, a suburb of Chomutov, Czech Republic (50°27′38" N, 13°23′07" E). The experimental field consisted of four replicates for each soil amendment applied, arranged in a completely randomized block design. Each plot measured 2.5 × 2.0 m and contained 30 rhizomes. The pit method was employed for planting. Prior to planting, various organic amendments were incorporated into the soil, including biochar at single (5.0%) and double (10.0%) doses (designated as BD1 and BD2), biogas digestate (D), sewage sludge (SS), and hemicellulose waste (HW).

The amendments were mixed with excavated soil, resulting in a total volume of approximately 3.5 liters. The excavated soil was transferred to a plastic bucket and manually mixed. Subsequently, the amendments were incorporated as detailed below:

– BD1 – a total of 180 mL of biochar with a dry matter content of approximately 97.0% was utilized;

– BD2 – a total of 360 mL of biochar with a dry matter content of approximately 97.0% was utilized;

– SS – a total of 1000 mL of sewage sludge with an approximate dry matter content of 38.0% was utilized;

– D – a total of 1000 mL of liquid biogas digestate with an approximate dry matter content of 29.0% was utilized;

– HW – a total of 1000 mL of paper sludge with a dry matter content of approximately 28.0% was utilized;

– No amendments were provided in the control group.

The additives were thoroughly mixed with the original soil, and approximately half of the resulting mixture was poured into the designated hole. Subsequently, the *Miscanthus* rhizome was inserted into the hole, and the remaining mixture of soil and additives was placed on top. The rhizomes, which were three years old, had an average weight of 20 ± 2 g. They were planted in both the amended soil and the control soil at a depth of 10–12 cm, with a spacing of 1 m between plots. The *M.* × *giganteus* rhizomes were initially transferred from the deposit field. Throughout the duration of the experiment, the research plots were not subjected to fertilization or irrigation. *Miscanthus* × *giganteus* biomass was harvested at the conclusion of the second vegetative season, specifically on November 15, 2022 (green harvest), and on March 15, 2023 (brown harvest). Soil samples for nematode isolation were collected from the *M.* × *giganteus* plantation three times during the second growing season, on April 12, June 9, and October 18, 2022. Additionally, on October 18, 2022, soil samples were obtained for microbiological analysis. Approximately 500 g of soil samples were collected from the root zone of *M.* × *giganteus* at a depth of 35 cm using a soil probe, with samples taken from four distinct locations for each replicate.

Soil characteristics. The agrochemical parameters of the soil were assessed prior to the establishment of the plantation, as described by Stefanovska et al. (2022). According to the classification by the Food and Agriculture Organization (FAO), the soil is categorized as a Cambisol (Schad, 2023). The evaluation of soil quality was conducted in accordance with the standards set forth in the Decree of the Ministry of Agriculture of the Czech Republic regarding agrochemical testing of agricultural soils and the determination of soil properties for forestland, No. 275/1998 Coll., as amended by the current edition No. 4 dated January 11, 2017. The soil was found to be strongly acidic, with a pH of 4.9 ± 0.2 in KCl, and moderately acidic, with a pH of 5.7 ± 0.2 in H_2O. The potassium (K) content was measured at 315 ± 9.8 mg/kg (optimal range: 171–310 mg/kg), the magnesium (Mg) content was 258 ± 7.3 mg/kg (optimal range: 61–265 mg/kg), and the phosphorus (P) content was 50.6 ± 2.0 mg/kg (optimal range: 86–125 mg/kg). The organic matter content of the soil was determined to be 4.6 ± 0.3%, which is classified as medium-high.

Nematode isolation and identification. Nematodes were isolated utilizing a centrifugation-flotation technique, which entails the addition of water to a soil sample, allowing the sediment to settle at the bottom (Szczygieł, 1971). The resulting sediment suspension was decanted and subjected to centrifugation at 2000 g for a duration of three minutes. The supernatant was then resuspended in 80 mL of a sucrose solution. Subsequently, the nematode-containing solution was filtered through a 25 mm sieve, and the nematodes were extracted and transferred to glass containers. Following extraction, the nematodes were killed and fixed in TAF (a solution comprising 8.0% formalin and 2.0% triethanolamine in distilled water). They were then subjected to a series of glycerol-ethanol solutions before being stored in anhydrous glycerol on slides (Seinhorst, 1966). After a 24-hour period for alcohol evaporation, the fixation dish was placed in a desiccator containing silica gel to facilitate the evaporation of any remaining traces of water. A total of one hundred individuals, initially identified per sample, were classified as species (plant-parasitic nematodes) or genera of free-living nematodes using a Zeiss Jena A-Scope microscope, with identification keys referenced from Brzeski (1998) and Andrássy (2007).

Nematode fauna analysis and the calculation of ecological indexes. The nematode community was analyzed by assessing the total number of nematodes and their relative proportions within the soil samples. The soil nematodes were categorized into five trophic groups, as outlined by Yeates et al. (1993). Each taxon was classified into one of five colonizer-persister classes, where "persister" nematodes are indicative of stability and complexity within the food web, while "colonizer" nematodes reflect resource availability. The maturity index (MI) for free-living nematodes and the plant parasite index (PPI) for herbivorous nematodes were computed based on the ratio of a specific genus to the total nematode population, along with their assigned c–p values (Bongers, 1990). Additional indices reflecting the structure and complexity of the soil food web, as described by Ferris et al. (2001) included the channel index (CI), basal index (BI), enrichment index (EI), and structure index (SI). The SI serves as an indicator of the complexity of the soil food web in response to soil disturbance, whereas the CI and EI provide insights into the decomposition of organic matter. The evaluation of the soil food web was conducted through a scatter plot analysis of SI versus EI values. The ecological indices were calculated utilizing the Nematode Indicator Joint Analysis methodology (Sieriebriennikov et al., 2014).

Microbiological analysis. The soil samples were meticulously mixed and sieved to eliminate stones and larger particles. Subsequently, 10 grams of the sieved soil were combined with 100 milliliters of a 0.85% NaCl aqueous solution and agitated in a rotary shaker for 20 minutes. The soil dilution plating method was employed to quantify the populations of various microbial groups on selective media. The total bacterial count, including actinomycetes, was assessed using soil extract media. The enumeration of fluorescent *Pseudomonas* bacteria was conducted on S1 media under ultraviolet light (Gould et al., 1985). Additionally, Rose Bengal medium (Martin, 1950) was utilized to quantify soil fungi. The microbial populations were expressed as colony-forming units (CFU) per gram of dry soil weight.

Enzyme dehydrogenase activity. Dehydrogenase activity (DHA) was assessed following the Cassidy procedure as outlined by Alef and Nannipieri (1995), and Brzezińska and Włodarczyk (2005). A total of 3 grams of sieved soil was placed in a 15 mL dark tube to minimize light exposure. Subsequently, 1.8 mL of sterile deionized water, 600 µL of a 1.0% glucose suspension, and 600 µl of a 3.0% aqueous solution of 2,3,5-triphenyltetrazolium chloride (TTC) were added to the tube. The mixture was then incubated for 24 hours at 30 °C in the dark. During the incubation period, TTC was enzymatically reduced to form water-insoluble 1,3,5-triphenylformazane (TPF). The reaction was terminated by the addition of 12 mL of 96.0% ethanol, and the mixture was agitated in the dark for one hour. Subsequently, the samples were centrifuged at 12,000 rpm 8 min minutes 4 °C. The supernatant was utilized for spectrophotometric measurements of the TPF concentration at a wavelength of 485 nm. The measured values were compared to a standard curve established with known TPF concentrations. Ultimately, the mean dehydrogenase activity was quantified in terms of dehydrogenase activity units, representing the amount of TPF produced per gram of soil over a 24-hour period [µmol TPF/g s.m. 24 h].

Statistical analysis. The descriptive statistics and General Linear Models were computed using the STATISTICA Data Analysis Software System (StatSoft Inc., Palo Alto, CA, USA, Version 12.0, 1984–2014). Basic statistics (means, medians, correlations, linear regressions, and standard deviations) were evaluated followed by inferential statistics (such as ANOVA and multiple comparisons of means).

Results

Structure of the nematode communities. The nematode community was comprised of 25 species belonging to five distinct trophic groups: herbivores, bacterivores, fungivores, omnivores, and predators. These species were further classified into ecological strategies as either free-living or plant-parasitic nematodes (Table 1).

Table 1
Species diversity of the nematode community, ecological characteristics of nematodes, average nematode abundance, and variation in abundance described by the influence of amendments

Nematode group	Species	C–p class	P–p class	Feeding type	Mass, µg	Mean ± SD	R_{adj}^2	P
Plant parasitic	*Paratylenchus projectus* Jenkins, 1956	–	2	Plant-parasitic nematodes – ectoparasites	0.05	20.5 ± 21.8	0.47	< 0.001
	Geocenamus quadrifer (Andrássy, 1954) Brzeski, 1991	–	3	Plant-parasitic nematodes – ectoparasites	0.29	12.8 ± 13.4	0.60	< 0.001
	Merlinius nothus (Allen, 1955) Siddiqi, 1970	–	3	Plant-parasitic nematodes – ectoparasites	0.20	13.7 ± 16.4	0.65	< 0.001
	Merlinius joctus (Thorne, 1949) Sher, 1974	–	3	Plant-parasitic nematodes – ectoparasites	0.21	9.9 ± 13.0	0.63	< 0.001
	Helicotylenchus digonicus Perry, 1959	–	3	Plant-parasitic nematodes – semi–endoparasites	0.23	16.5 ± 21.6	0.58	< 0.001
	Helicotylenchus pseudorobustus (Steiner, 1914) Golden, 1956	–	3	Plant-parasitic nematodes – semi–endoparasites	0.29	18.6 ± 21.0	0.47	< 0.001
	Helicotylenchus vulgaris Yuen, 1964	–	3	Plant-parasitic nematodes – semi–endoparasites	0.51	8.8 ± 12.9	0.64	< 0.001
	Pratylenchus fallax Seinhorst, 1968	–	3	Plant-parasitic nematodes – migratory endoparasites	0.08	18.9 ± 17.1	0.72	< 0.001
	Pratylenchus thornei Sher & Allen, 1953	–	3	Plant-parasitic nematodes – migratory endoparasites	0.13	21.5 ± 17.0	0.64	< 0.001
Free living	*Acrobeloides* sp.	2	–	Bacterivores	1.26	26.1 ± 22.5	0.80	< 0.001
	Cephalobus sp.	2	–	Bacterivores	0.27	24.7 ± 15.0	0.50	< 0.001
	Panagrolaimus sp.	1	–	Bacterivores	0.66	18.7 ± 13.4	0.61	< 0.001
	Plectus sp.	2	–	Bacterivores	0.86	19.6 ± 16.8	0.45	< 0.001
	Rhabditis sp.	1	–	Bacterivores	7.50	28.6 ± 28.5	0.67	< 0.001
	Aphelenchoides sp.	2	–	Fungivores	0.15	27.0 ± 22.6	0.82	< 0.001
	Aphelenchus sp.	2	–	Fungivores	0.22	84.0 ± 55.4	0.82	< 0.001
	Ditylenchus sp.	2	–	Fungivores	0.50	35.4 ± 30.9	0.84	< 0.001
	Filenchus sp.	2	–	Fungivores	0.10	42.1 ± 20.2	0.80	< 0.001
	Dorylaimus sp.	4	–	Omnivores	42.77	37.1 ± 29.3	0.91	< 0.001
	Eudorylaimus sp.	4	–	Predators	3.19	20.6 ± 15.7	0.68	< 0.001
	Mesodorylaimus sp.	4	–	Omnivores	1.28	11.1 ± 11.8	0.64	< 0.001
	Coomansus sp.	4	–	Predators	6.32	7.4 ± 4.9	0.35	0.020
	Iotonchus sp.	4	–	Predators	7.34	3.4 ± 5.8	0.54	< 0.001
	Mylonchulus sp.	4	–	Predators	1.76	10.6 ± 9.9	0.63	< 0.001
	Prionchulus sp.	4	–	Predators	11.59	8.3 ± 10.3	0.55	< 0.001

Among the plant-parasitic nematodes, nine species were identified, including four ectoparasitic species, three semi-endoparasitic species, and two migratory endoparasitic species. The community included five genera of bacterivores, three genera of fungivores, five genera of predators, and two genera of omnivores. The proportion of plant-parasitic nematodes relative to the total nematode abundance was significantly affected by the type of amendment applied during the growing season (F = 24.1, P < 0.001, Table 2). The highest proportion of plant-parasitic nematodes within the community was observed in April (28.8 ± 1.8%), followed by a slight decrease in June (22.8 ± 1.9%), and the lowest proportion was recorded in October (21.4 ± 2.4%). The relationship between herbivores and total community abundance exhibited variability under the influence of BD1 or SS (Planned comparison F = 2.6, P = 0.11).

Table 2

Assessment of the soil ecosystem using nematode-based indices (ANOVA P-value <0.001 foe all parameters)*

Parameter	Index name	Control	BD 1	BD 2	D	HW	SS
Community biomass and abundance	Total biomass, mg	1.17 ± 0.24^{a}	1.66 ± 0.33^{b}	2.87 ± 0.68^{c}	1.87 ± 0.37^{b}	4.54 ± 0.91^{d}	0.82 ± 0.11^{e}
	Total number, ind.	560.6 ± 43.8^{a}	482.3 ± 23.1^{b}	1194.8 ± 285.9^{c}	318.8 ± 29.9^{e}	398.0 ± 26.3^{e}	318.1 ± 28.8^{e}
Community maturity	Maturity index	2.251 ± 0.041^{a}	2.653 ± 0.124^{b}	2.972 ± 0.110^{c}	2.374 ± 0.052^{a}	2.392 ± 0.031^{a}	2.390 ± 0.064^{a}
	Maturity index 2–5	2.35 ± 0.091^{a}	2.860 ± 0.130^{b}	3.09 ± 0.113^{c}	2.48 ± 0.064^{a}	2.56 ± 0.062^{d}	2.55 ± 0.091^{d}
	Sigma maturity index (SMI)	2.442 ± 0.043^{a}	2.691 ± 0.101^{b}	2.942 ± 0.102^{c}	2.513 ± 0.054^{a}	2.562 ± 0.033^{a}	2.442 ± 0.112^{a}
	Plant parasitic index (Plant-parasitic nematodes)	2.951 ± 0.022^{a}	2.856 ± 0.034^{a}	2.668 ± 0.235^{b}	2.951 ± 0.032^{a}	2.867 ± 0.014^{a}	2.591 ± 0.223^{b}
Community functions	Channel index (CI)	69.4 ± 11.0^{a}	41.6 ± 3.4^{b}	43.2 ± 4.9^{b}	64.7 ± 9.4^{a}	52.1 ± 6.6^{c}	56.2 ± 24.7^{c}
	Basal index (BI)	32.3 ± 3.9^{a}	18.1 ± 3.2^{b}	15.0 ± 2.5^{b}	29.7 ± 2.6^{c}	25.2 ± 6.1^{c}	26.1 ± 4.7^{c}
	Enrichment index (EI)	55.5 ± 2.5^{a}	60.5 ± 5.3^{a}	47.9 ± 2.4^{b}	52.6 ± 3.1^{a}	58.7 ± 2.1^{a}	57.6 ± 6.8^{a}
	Structure index (SI)	45.7 ± 7.5^{a}	74.8 ± 4.7^{b}	82.5 ± 3.4^{b}	55.4 ± 3.8^{a}	60.5 ± 3.5^{c}	60.0 ± 5.7^{c}
Footprint	Composite	216 ± 42^{a}	304 ± 41^{b}	660 ± 121^{c}	281 ± 44^{b}	564 ± 148^{c}	159 ± 25^{d}
	Enrichment	52.1 ± 15.1^{a}	49.7 ± 12.8^{a}	33.7 ± 7.6^{b}	46.3 ± 11.4^{a}	159.6 ± 61.0^{c}	44.1 ± 19.8^{a}
	Structure	140 ± 27^{a}	236 ± 49^{b}	602 ± 123^{c}	204 ± 42^{b}	330 ± 69^{d}	99 ± 10e
	Herbivore (Plant-parasitic nematodes)	12.73 ± 1.7^{a}	5.95 ± 1.71^{b}	3.18 ± 1.58^{b}	9.92 ± 2.37^{c}	39.24 ± 11.6^{d}	5.21 ± 2.45^{b}
	Fungivore	26.26 ± 5.15^{a}	7.71 ± 1.09^{b}	6.09 ± 1.34^{b}	17.5 ± 2.43^{c}	38.32 ± 5.95^{d}	13.54 ± 3.41^{e}
	Bacterivore	36.72 ± 20.7^{a}	54.41 ± 15.52^{b}	48.73 ± 7.01^{a}	49.42 ± 15.26^{a}	155.97 ± 65.44^{c}	40.99 ± 25.6^{a}
	Predator	31.25 ± 11.29^{a}	50.71 ± 14.28^{b}	79.28 ± 12.93^{c}	36.87 ± 7.84^{a}	132.8 ± 42.43^{d}	41.62 ± 10.3^{a}
	Omnivore	109.15 ± 20.92^{a}	185.56 ± 62.11^{b}	523.61 ± 113.05^{c}	167.83 ± 42.05^{d}	197.74 ± 35.95^{d}	57.75 ± 9.22^{e}
Trophic groups, % from the total community abundance	Herbivore (Plant-parasitic nematodes)	26.98 ± 3.62^{a}	24.40 ± 5.83^{a}	36.89 ± 1.95^{b}	24.02 ± 7.04^{a}	10.48 ± 3.00^{c}	23.34 ± 5.10^{a}
	Fungivore	48.13 ± 6.72^{a}	38.11 ± 2.67^{b}	30.08 ± 3.75^{c}	24.22 ± 1.67^{d}	15.40 ± 3.45^{e}	35.79 ± 8.16^{b}
	Bacterivore	12.99 ± 4.30^{a}	20.74 ± 4.77^{b}	17.41 ± 1.56^{b}	23.01 ± 7.45^{b}	28.11 ± 3.79^{c}	21.99 ± 10.11^{b}
	Predator	5.76 ± 2.08^{a}	7.88 ± 1.90^{b}	10.32 ± 0.89^{c}	16.47 ± 1.92^{d}	20.97 ± 1.89^{e}	14.54 ± 3.68^{d}
	Omnivore	6.18 ± 0.89^{a}	8.88 ± 1.01^{b}	5.29 ± 0.70^{a}	12.24 ± 3.15^{c}	25.01 ± 3.50^{d}	4.32 ± 1.03^{e}
Plant parasitic nematodes	Migratory endoparasites, % of herbivores	41.62 ± 2.37^{a}	34.21 ± 5.14^{b}	18.37 ± 15.53^{c}	39.56 ± 1.74^{a}	22.76 ± 1.68^{c}	20.00 ± 11.95^{c}
	Semi-endoparasites, % of herbivores	29.14 ± 3.77^{a}	27.29 ± 3.58^{a}	36.31 ± 7.11^{b}	27.42 ± 2.42^{a}	35.54 ± 1.99^{b}	14.88 ± 6.81^{c}
	Ectoparasites, % of herbivores	29.21 ± 3.26^{a}	38.50 ± 6.78^{b}	45.32 ± 14.67^{c}	32.99 ± 3.47^{a}	41.70 ± 1.45^{b}	65.11 ± 16.79^{d}

Note: average values that have the same letter are not statistically different (P > 0.05; planned comparison test).

In soils treated with BD2 (higher dose), a decrease in the proportion of herbivores within the community was noted (planned comparison F = 106.6, P < 0.001). The application of D resulted in a significant increase in the abundance of plant-parasitic nematodes (Planned comparison F = 38.5, P < 0.001). The proportion of migratory endoparasites among herbivores declined during the vegetative season, from 32.9 ± 2.0% to 25.6 ± 3.7% (F = 3.8, P = 0.03). The application of BD2 significantly reduced the proportion of migratory endoparasites to 22.76 ± 0.56%. Similar effects were observed with the D and SS treatments (F = 15.2, P < 0.001). The proportion of semi-endoparasites did not exhibit statistically significant changes throughout the vegetative season (F = 2.3, P = 0.11). The most pronounced decrease in this group of plant-parasitic nematodes was observed under the SS treatment, with a proportion of 14.8 ± 2.2% (Planned comparison F = 93.9, P < 0.001). Conversely, the proportion of ectoparasites increased during the vegetative season, rising from 36.9 ± 1.7% to 45.8 ± 4.4% (F = 4.7, P = 0.013). This group of plant-parasitic nematodes experienced a decline in abundance due to the influence of organic amendments, with the most significant effects noted following the application of BD1 (32.9 ± 1.2%) and SS (38.5 ± 2.2%) (F = 17.6, P < 0.001).

Functional state of nematode communities. The condition of the soil system was evaluated utilizing nematode-based indices. The nematode community exhibited an abundance of 560.56 ± 43.79 individuals per sample in the control soil. The application of amendment BD1 did not significantly alter the community's abundance (planned comparison F = 2.61, P = 0.15) when compared to the control. Conversely, the application of other amendments, with the exception of BD2, resulted in a reduction of community abundance to 322.81 ± 65.38 individuals per sample (planned comparison F = 24.37, P < 0.001). Notably, an increase in community abundance was recorded following the application of BD2, reaching 1194.78 ± 40.04 individuals per sample (planned comparison F = 125.43, P < 0.001). In the control soil, the community biomass was measured at 1.17 ± 0.24 mg per sample. The application of SS did not significantly affect the community's biomass in comparison to the control (planned comparison F = 2.1, P = 0.15), whereas other organic amendments contributed to an increase in biomass (planned comparison F = 135.7, P < 0.001). HW exhibited the most pronounced stimulatory effect on the biomass of the nematode community. The incorporation of amendments resulted in an increase in the maturity index (MI) (F = 102.6, P < 0.001), with biochar demonstrating the most substantial effect on MI, showing a positive correlation with dosage. The variability patterns of MI 2–5 and Sigma MI were analogous to those of MI. Amendments D and BD1 did not significantly influence the PPI (Planned comparison F = 1.88, P = 0.18), while BD1 and SS were found to reduce the PPI (planned comparison F = 11.9, P < 0.001). The incorporation of amendments led to a decrease in the community index (CI) (F = 7.5, P < 0.001), with biochar having the most significant impact on this index. The basal index (BI) exhibited a similar response to the amendments (F = 35.6, P < 0.001). Amendments D and SS did not significantly affect the enrichment index (planned comparison F = 2.2, P = 0.14), whereas D and BD1 stimulated an increase in this index (planned comparison F = 11.9, P < 0.001). The application of BD2 resulted in a decrease in the enrichment index (planned comparison F = 21.3, P < 0.001). The application of amendments led to an increase in the structure index (SI) (F = 64.3, P < 0.001), with the most pronounced effect observed in soil treated with biochar. Furthermore, the application of amendments increased the composite footprint across all treatments (planned comparison F = 56.7, P < 0.001), with the exception of SS, which did not significantly affect the composite footprint (planned comparison F = 2.1, P = 0.16). The use of amendment D stimulated an increase in nematodes, which are the most responsive to resource enrichment, as evidenced by an increase in the enrichment footprint. BD2 was associated with the largest increase in the structure footprint, indicating an enhancement in the regulatory functions of the nematode community.

Nematode community food web analysis. The analysis of nematode community distribution within the C-P (colonizer-persister) triangle revealed that the overall condition of all nematode communities can be classified as stressful, characterized by low levels of enrichment. Notably, the control plots exhibited the most pronounced stress conditions. The application of amendments resulted in a reduction of stress within the soil ecosystem, accompanied by a modest increase in enrichment in certain instances. The most significant stabilizing effect was observed following the application of biochar, which demonstrated a dose-dependent relationship. Furthermore, the introduction of amendments served as a catalyst for alterations within the trophic network. Based on the degree of impact of the amendments on the nematode community, the effects can be ranked as follows: BD2 → BD1 → D → SS → HW.

Microbiological responses to soil amendments. The application of organic amendments to marginal soils under *M.* × *giganteus* cultivation did not generally influence the microbial groups examined in this study. An exception was observed in the case of fluorized *Pseudomonas* bacteria, whose abundance significantly increased in the soil treated with BD2 (Table 3).

Table 3
Microbiological analysis of soil sampled at the marginal land (n = 3, mean ± SD)

Treatment	Fluorized *Pseudomonas* CFU x 10^5 / g	Bacteria CFU x 10^7 / g	Actinomycetes CFU x 10^6 / g	Filamentous fungi CFU 10^5 / g	F:B^a	Dehydrogenase activity mmol TPF/g dry weight of soil/24 hr
Control	1.58 ± 0.33^b	2.65 ± 1.00^b	5.94 ± 1.69^a	1.96 ± 0.73^a	0.00712 ± 0.00452^b	3.83 ± 0.22
BD1	1.51 ± 0.12^b	3.13 ± 1.46^a	5.22 ± 3.28^b	0.74 ± 0.20^b	0.00274 ± 0.00104^b	3.10 ± 0.67
BD2	2.85 ± 0.19^a	2.67 ± 0.21^b	6.81 ± 5.26^b	1.08 ± 1.01^b	0.00332 ± 0.00206^b	3.84 ± 1.23
HM	1.59 ± 0.11^b	2.26 ± 0.46^b	3.08 ± 0.89^b	0.80 ± 0.34^b	0.00382 ± 0.00196^b	4.76 ± 0.84
SS	1.75 ± 0.48^b	1.59 ± 0.59^b	5.15 ± 3.83^b	1.04 ± 0.89^b	0.00542 ± 0.00237^b	4.30 ± 0.33
D	1.02 ± 0.60^b	2.39 ± 0.92^b	7.79 ± 2.87^b	0.95 ± 0.58^b	0.00312 ± 0.00186^b	3.38 ± 0.39

Notes: F:B is the ratio of bacteria (including actinomycetes) to fungi; the values indicated by different letters are significantly different; the letter "a" indicates the highest value; the numbers in columns without letters did not differ significantly.

The concentration of these bacteria in the control soil was measured at 1.58×10^5 CFU/g, whereas in the BD2 amended soil, the concentration rose to 2.85×10^5 CFU/g. The abundance of *Pseudomonas* in the other treatment groups did not differ significantly from that in the control group. Furthermore, the application of organic amendments was found to significantly enhance the relationship between bacteria and fungi by increasing the contribution of bacteria to the microbial community, while the proportion of fungi decreased. The F:B index in the control soil was 0.007, which was significantly higher than the average value of 0.003 observed in the other treatments. This finding was corroborated by fungal enumeration data, which indicated that the highest number of fungi was isolated from non-amended soil; however, this difference was not statistically significant when compared to the amended samples. The overall number of bacteria, including actinomycetes, did not exhibit significant variation among the treatments. Additionally, the enzyme dehydrogenase activity ranged from 3.10 to 4.76 TFP/g of dry weight of the soil and did not show significant differences across the treatments.

Correlation between nematode community indices and microbiological parameters. The results presented in Table 4 indicate that the abundance of fluorized *Pseudomonas* exhibited a negative correlation with the overall abundance of nematode communities, as well as with the PPI and EI metrics. Additionally, it was negatively correlated with the footprints of herbivores, fungivores, bacterivores, and predators, while demonstrating a positive correlation with the MI, SI, and omnivore footprints. Furthermore, the abundance of bacteria in the soil was positively correlated with Sigma MI and the omnivore footprint. The abundance of actinomycetes showed a positive correlation with the total number and biomass of the nematode community, Sigma MI, composite footprint, structure, and both predator and omnivore footprints. The quantity of fungi was positively correlated with the CI. The F:B index was positively correlated with the CI and negatively correlated with the SI. Lastly, dehydrogenase activity was found to be negatively correlated with enrichment, bacterivore, and predator footprints.

Table 4
The correlation between nematode community indices and soil microbiology parameters
(correlation coefficients that are significant are presented only at $P < 0.05$)

Parameter	Index	Fluorized *Pseudomonas*, CFU x 10^5/g	Bacteria, CFU x 10^7/g	Actinomycetes, CFU x 10^6/g	Filamentous fungi, CFU 10^5/g	F:B^a	Dehydrogenase activity, mmol TPF/g dry weight of soil/ 24 hr
Community biomass and abundance	Total biomass, mg	–	–	0.712	–	–	–
	Total number, ind.	–0.712	–	0.501	–	–	–
Community maturity	Maturity index (MI)	0.647	–	–	–	–	–
	Maturity index 2–5 (MI)	0.562	–	–	–	–	–
	Sigma maturity index (SMI)	–	0.378	0.576	–	–	–
	Plant parasitic index (PPI)	–0.643	–	–	–	–	–
Community functions	Channel index (CI)	–	–	–	0.324	0.379	–
	Basal index (BI)	–	–	–	–	–	–
	Enrichment index (EI)	–0.610	–	–	–	–	–
	Structure index (SI)	0.477	–	–	–	–0.372	–
Footprint	Composite footprint	–	–	0.742	–	–	–
	Enrichment footprint	–0.775	–	–	–	–	–0.301
	Structure footprint	–	–	0.682	–	–	–
	Herbivore footprint	–0.763	–	–	–	–	–
	Fungivore footprint	–0.776	–	–	–	–	–
	Bacterivore footprint	–0.742	–	–	–	–	–0.304
	Predator footprint	–0.504	–	0.607	–	–	–0.304
	Omnivore footprint	0.551	0.272	0.572	–	–	–

Note: * – ratio of bacteria (including actinomycetes) to fungi.

Miscanthus yield. The yield obtained from the green harvest in November 2022 was significantly greater than that from the brown harvest in March 2023, as indicated by a planned comparison (F = 3.31, P = 0.05). A significant correlation was observed between the fresh weight of harvested *Miscanthus* biomass and the quantity of organic amendments incorporated into the soil. The application of biochar resulted in a substantial increase in *M.* × *giganteus* yield, as demonstrated by a planned comparison (F = 45.8, P < 0.001), with this effect being dose-dependent. Biochar positively influenced the yield at both harvest periods. Conversely, the addition of HW led to a reduction in biomass, as evidenced by a planned comparison (F = 4.9, P = 0.03). The incorporation of D and SS did not have a significant impact on *M.* × *giganteus* yield during the second year of plantation growth. Furthermore, the dry matter content exhibited significant variation across the different harvest dates (F = 466.0, P < 0.001), with the dry matter content being higher for biomass harvested in March 2023 (50.6 ± 0.8%) compared to that harvested in November (34.3 ± 0.8%). The amendments did not significantly influence the variation in dry matter content of the biomass (F = 0.13, P = 0.99). Additionally, *M.* × *giganteus* yield was found to be negatively correlated with the number of herbivores (r = –0.87, P < 0.001), with the reduction of migratory endoparasites being the most significant contributor to yield enhancement (r = –0.87, P < 0.001). The reduction of ectoparasites had a lesser impact on yield (r = –0.69, P < 0.001), while the smallest effect was attributed to changes in the proportion of semi-endoparasites (r = –0.46, P = 0.024).

Discussion

Structure of nematode communities. Soil organic amendments have the potential to enhance various soil properties, including structure, temperature, humidity, and nutrient availability during plant growth. Additionally, these amendments can modify the soil microflora and microfauna, particularly affecting soil nematodes. Such alterations may lead to an increase in beneficial nematode populations while simultaneously reducing the abundance of economically significant plant-parasitic nematodes, thereby influencing plant growth and nutrient uptake (Renčo, 2013). The present study demonstrated that the application of biochar resulted in a decrease in PPN abundance. This finding aligns with the results reported by Cole et al. (2021), who found that an 8.0% biochar application did not significantly alter total nematode abundance but did reduce the abundance of plant-parasitic nematodes. A plausible explanation for this phenomenon is that biochar treatment may enhance plant defense mechanisms, thereby reducing the likelihood of nematode infection through modifications in nematode population biodiversity (Poveda et al., 2021). These results are consistent with those of Rahman et al. (2014), who observed a reduction in plant-parasitic nematodes populations following the addition of biochar derived from poultry litter in vineyards, which was accompanied by an increase in free-living nematodes. Zang et al. (2013) reported a decrease in plant-parasitic nematodes from the genera *Coslenchus*, *Hirschmanniella*, *Rotylenchus*, and *Tylenchus* as a result of applying biochar produced from wheat straw at doses ranging from 2.4 to 48 t/ha. Eche et al. (2021) indicated that a mixed formulation of sawdust and eucalyptus biochar moderated the beneficial effects of plant-parasitic nematodes. However, it is important to note that the impact of biochar on plant-parasitic nematodes is dose-dependent. In higher doses (10%), biochar treatment BD2 resulted in a decrease in PPN abundance, whereas in lower doses (5%), treatment BD1 did not significantly affect this nematode group. Furthermore, the abundance of nematodes remained unchanged with the application of SS and HW. The dose-dependent effects of biochar on the nematode community have also been documented by Domene et al. (2021). Nevertheless, this research did not corroborate the notion of a negative impact of biochar on plant-parasitic nematodes, indicating that lower doses may promote the presence of predators and plant-parasitic nematodes, while higher application rates tend to favor bacterivores and fungivores.

The findings regarding the specific effects of biochar and other amendments on various groups within plant-parasitic nematodes clearly indicate two primary outcomes. Firstly, migratory endoparasites, specifically *Pratylenchus* spp., exhibited the highest sensitivity to the incorporation of these amendments. The application of BD1 and BD2 resulted in the most significant reduction of this nematode population, followed by the application of SS and D. Secondly, the application of BD2 and SS effectively suppressed the populations of ectoparasites, including *Paratylenchus projectus*, *Geocenamus quadrifer*, *Merlinius nothus*, and *Merlinius joctus*. These fundamental findings align with previous research indicating that a 5% biochar derived from spelt husk and coniferous wood significantly decreased the infection rates of the nematode *Pratylenchus penetrans* in carrot fields (George et al., 2016). Furthermore, Poveda et al. (2021) reported that biochar was effective in reducing populations of *Pratylenchus coffeae*, a migratory endoparasitic nematode that causes substantial root lesions in banana plants. Additionally, Rahayu & Puspita Sari (2017) conducted an *in vitro* experiment demonstrating that the application of 4% woody biochar significantly diminished populations of the parasitic nematode *Pratylenchus coffeae*. The use of biochar derived from poultry litter was also found to significantly reduce the populations of *Meloidogyne javanica*, *Tylenchulus semipenetrans*, *Pratylenchus* spp., *Helicotynchus* spp., and *Criconemoid* spp. in grapevines, while simultaneously enhancing the diversity of beneficial plant-associated organisms (Rahman et al., 2014).

The findings presented herein provide confirmatory evidence that biochar positively influences nematode community biomass, with the most significant increases observed in BD2 and HW. This observation aligns well with previous research indicating that the addition of organic matter results in an increase in nematode community biomass (Andriuzzi & Wall, 2018). A similar trend was noted regarding nematode abundance and the functional characteristics of nematode assemblages. Our results indicate that soil nematode communities are influenced by variations in carbon input. The incorporation of biochar, which is characterized by a high carbon content, likely enhances the concentration of soil organic carbon (SOC). This effect may be particularly pronounced in the *M.* × *giganteus* plantation, as this crop is classified as a C-4 type (Agostini et al., 2015). Numerous studies have documented that *M.* × *giganteus* possesses significant potential for enhancing SOC stocks through the capture and allocation of carbon belowground (Felten & Emmerling, 2012). A comprehensive investigation is warranted to examine how nematode communities, as integral components of the soil food web, respond to increasing SOC levels under long-term *M.* × *giganteus* phytotechnologies aimed at biomass production. SOC serves as a growth substrate for microorganisms, which are preyed upon by bacterivorous and fungivorous nematodes (van den Hoogen et al., 2019). The observed increase in nematode community biomass in conjunction with rising SOC levels may be attributed to a more complex food web that supports a higher proportion of large-bodied, higher-trophic-level nematodes (Verschoor et al., 2001).

Functional state of nematode communities. Our findings demonstrate that the assessment of soil nematode diversity, based on various taxonomic levels and functional groups, serves as an effective ecological tool for analyzing the impacts of anthropogenic activities that induce disturbances, such as soil amendments (Du Preez et al., 2022). The maturity index (MI) is frequently employed to evaluate environmental disturbances through the analysis of nematode species composition. In our investigation, the MI values observed in the amendment treatments ranged from 2.37 to 2.97, suggesting that these soil conditions were situated within a stage of ecological succession. Soil amendments, in conjunction with other disturbances such as cultivation, pollution, and the application of fertilizers, facilitate the initial stages of ecological succession within an ecosystem. All amendments tested resulted in alterations to the nematode community, which were accompanied by an increase in MI compared to the control soil. The influence of the amendments on the MI value was primarily attributed to the heightened presence of opportunistic bacterivorous nematodes, particularly *Rhabditis*, which exhibit rapid reproduction and respond to bacterial availability when nutrient levels are elevated (Hu & Qi, 2010). This conclusion aligns with the findings of Cole et al. (2021) and Shi et al. (2023), who reported an increase in MI with the addition of biochar compared to untreated plots. In our study, the effect of biochar on the MI was dose-dependent; specifically, the application of BD2 at a relatively high dosage resulted in a correspondingly high MI value. Conversely, Van Sinh et al. (2022) reported that the highest MI was observed at a biochar dosage of 5 Mg/ha, while the lowest values were recorded at a dosage of 40 Mg/ha. In the second year of our observations, the application of SS resulted in a 20% increase in MI compared to BD2, which contrasts with our findings from the first year of the *Miscanthus × giganteus* vegetation study. The highest MI was associated with the application of SS, whereas the results for BD1 and BD2 were inconclusive.

The channel index (CI) exhibited significant variation with the application of soil amendments. In the control group, the CI indicated that the pathways of organic matter decomposition were predominantly influenced by fungal activity. This observation is corroborated by the analysis of microbial group relationships within the studied soils, where the control soil demonstrated the highest fungal-to-bacterial (F:B) ratio, suggesting a greater proportion of fungi in the total microbial population compared to soils treated with organic amendments. Across all treatments, the CI generally decreased relative to the control, with treatment D exhibiting the most pronounced reduction. For both doses of biochar derived from dairy manure (BD), the CIs were recorded at less than 50, indicating a transition from fungal to bacterial pathways upon the addition of BD. The accelerated rate of nitrogen mineralization and decomposition, as compared to the fungal energy pathway, can be attributed to the bacterial energy processes that enhance the functionality of a healthy soil food web (Zhang et al., 2023). A similar conclusion was drawn by Liu et al. (2020), who reported that biochar produced from peanut shells and applied at a rate of 4.5 t/ha improved the nematode community, as evidenced by the CI results. In a study conducted by Kilowasid et al. (2014), four different doses of biochar derived from wood waste were evaluated, revealing no significant differences in CI among the various application levels, which aligns with our findings. The enrichment index (EI) values indicated comparable levels of food availability (e.g., labile organic carbon) and nutrient enrichment. The EI ranged from 47 to 57 for the amendments tested, suggesting moderate to good nutrient availability. In our study, the EI reached its highest value of 60 for the BD1 treatment. Conversely, BD2 recorded the lowest index at 47, indicating that a smaller dose of biochar did not contribute to nitrogen enrichment during the growing season.

Nematode community food web analysis. Our findings align with existing research indicating that organic amendments contribute to increased soil maturity, enhancement of the soil ecosystem's nitrogen content, a reduction in the carbon-to-nitrogen (C:N) ratio, an elevation in the role of bacteria within trophic chains, and an augmentation of the mechanisms regulating trophic processes (Pires et al., 2023). These processes are intricately linked to alterations in the utilization of soil carbon within trophic chains. Although biochar did not lead to an increase in nematode abundance, it did promote a greater diversity of various trophic groups compared to the control experiment. These results are partially consistent with the findings of Puissant et al. (2021), who noted that soil nematodes, particularly free-living bacterivores and omnivore-carnivores, benefit from the addition of exogenous organic materials (Puissant et al., 2021).

Microbiological response of soil to the incorporation of amendments. Numerous studies have demonstrated that the incorporation of organic materials into soil significantly influences the abundance and diversity of soil microbial communities (Cui et al., 2023). However, the trajectory of microbiome alterations is contingent upon various soil factors, as well as the composition, structure, and fraction of the organic amendments applied. In our investigations, the application of organic materials did not yield a significant impact on the abundance of general microbial groups, including bacteria, actinomycetes, and fungi. Notably, only the higher dosage of biochar BD2 resulted in a significant increase in the population of fluorized *Pseudomonas*, and this increase was positively correlated with the growth of *Miscanthus* biomass. *Pseudomonas* species are recognized for their roles as plant growth-promoting and biocontrol bacteria (Anuroopa et al., 2021). In many instances, the addition of organic matter to soil has been associated with an increase in the density of the genus *Pseudomonas* (Dignam et al., 2019; Tao et al., 2020). Furthermore, it has been established that *Pseudomonas*, a member of the Proteobacteria phylum, are predominant bacteria that respond to organic amendments (Tao et al., 2020). However, in the studies referenced, only the application of biochar influenced their population dynamics. According to Han et al. (2017) the relative abundance of *Pseudomonas* in biochar-treated soils was significantly higher than in soils devoid of biochar, particularly in continuous cotton cropping systems. Additionally, Yan et al. (Yan et al., 2023) reported a positive effect of biochar on the viability and biofilm formation of *Pseudomonas aeruginosa* in soil. Numerous authors have underscored that biochar enhances the growth of microorganisms, thereby increasing both the number and diversity of microbial communities (Huang et al., 2023; Li et al., 2023). The key mechanisms through which biochar influences soil microorganisms include the provision of a favorable habitat due to its pore structure, the enhancement of soil pH, the supply of essential nutrients, the mitigation of toxic effects through adsorption, and the modulation of microbial signaling molecules (Huang et al., 2023). However, Ajema (2018), posits that the interactions between biochar and soil microorganisms cannot be conclusively characterized as solely positive; rather, these effects are contingent upon various factors, including the precursors utilized for pyrolysis, the conditions under which this process occurs, the type of soil, and the application rate of biochar. In our research, the application of biochar was found to affect only *Pseudomonas* bacteria, with no significant impact observed on fungal populations or the enzymatic activity of soil microorganisms. Conversely, Wiedener & Glaser (2013) and Li et al. (2023) reported that biochar creates a conducive environment for fungal growth. Additionally, Vithanage et al. (2018) demonstrated that biochar enhances the activity of redox enzymes in the soil, specifically catalase and dehydrogenase.

Compared to the control treatment, all organic fertilizer treatments significantly reduced the fungal-to-bacterial (F:B) ratio, indicating an increased prominence of bacteria within these microbial communities. This finding aligns with the previously described results concerning the nematode community, which exhibited a shift in their trophic pathways from fungal to bacterial in the amended soils. Cui et al. (2023) emphasized that organic amendments can substantially alter microbial community structure, even in instances where overall microbial activity and biomass remain unchanged. A similar effect was observed in our studies involving biochar and other amendments. Notably, higher doses of biochar resulted in pronounced changes, evidenced by an increase in the abundance of fluorescent *Pseudomonas*. However, in the case of digestate (D), sewage sludge (SS), and hemicellulose waste (HW) utilized in the current research, none of the amendments significantly affected the abundance of the studied microbial groups or dehydrogenase activity when compared to the control. The influence of digestate is consistent with the findings of Karimi et al. (2022), who conducted a meta-analysis of 56 articles examining the impact of digestates on soil microbiota, concluding that biogas digestates were neutral for soil microorganisms in half of the reviewed cases. Conversely, hemicellulose wastes are known to be challenging to degrade; thus, it is strongly recommended that they undergo pretreatment and composting prior to application (Khan et al., 2023). Following composting, these organic materials become more beneficial for soil functionality and microbial activity. Sewage sludge exhibits dual effects: on one hand, the organic matter and nutrients it contains enhance soil fertility and physical properties; on the other hand, the heavy metals present in sludge may diminish soil microbiome diversity, thereby promoting the proliferation of metal-resistant populations. Bacteria tend to be more sensitive to the application of sewage sludge, which may lead to an increase in the F:B ratio in cases of contamination (Khan & Scullion, 2000). This observation may elucidate the decrease in bacterial numbers noted upon the addition of sewage sludge compared to the control and other treatments, as well as the observed increase in the F:B ratio. These trends, however, did not reach statistical significance. Further longitudinal studies are warranted to enhance the understanding of these relationships.

Miscanthus yield. The findings of this study indicate that the yield of *M. × giganteus* from the green harvest was superior to that from the brown harvest, aligning with existing literature (Larsen et al., 2014; Bilandžija et al., 2018). Generally, the application of organic amendments is known to enhance the yields of energy crops by promoting nutrient uptake and facilitating oxidative decomposition (Luo et al., 2018). However, the results of the current investigation revealed that among the four amendments evaluated, only B1 and B2 resulted in an increase in *M.* × *giganteus* yield, while the application of amendment D led to a decrease in plant yield compared to the control group, which contradicts previously published findings (Dubis et al., 2022; Gurmessa et al., 2024). Furthermore, the effects of amendments B1, B2, and D on *M.* × *giganteus* yield during the second year of observation differed from those observed in the first growing season, where the incorporation of all amendments did not significantly influence *M.* × *giganteus* yield relative to the control (Stefanovska et al., 2022). In the long term, organic amendments have the potential to enhance plant yields, as their effects on crop productivity can persist for several years (Diacono & Montemurro, 2011). Numerous studies have demonstrated the long-term advantages of organic amendments. For instance, soils treated with sludge exhibited significantly higher yields of corn, wheat, barley, and rapeseed compared to control soils (Siebielec et al., 2018). The addition of sewage sludge and digestate to *M. sacchariflorus* was found to increase dry matter yield by 15% relative to the control treatment (Dubis et al., 2022). However, increasing the quantities of sewage sludge and digestate did not yield significant effects. Additionally, the incorporation of digestate, composted digestate, and digestate co-composted with food processing waste was shown to enhance sunflower yield (Gurmessa et al., 2024). Nevertheless, no significant differences were observed between the control treatment and other treatments, including those involving digestate co-composted with poultry litter and maize silage, as well as digestate co-composted with maize silage and poultry litter.

The observed increase in *M.* × *giganteus* plant yield attributable to the incorporation of biochar aligns with findings from existing field studies documented in the literature. Specifically, plots treated solely with biochar demonstrated the highest dry matter yield (*M.* × *giganteus*) during the second year of cultivation when compared to those treated with alternative fertilizers, such as biomass ash and a combination of biochar and biomass ash (Saletnik et al., 2018). The application of biochar has been shown to enhance the growth of *M.* × *giganteus* on marginal lands by 20–88% (Kharytonov et al., 2020). In comparison to other organic amendments, biochar is effective in retaining and making nutrients accessible to plants, thereby positively influencing plant yield. This efficacy is attributed to its extensive surface area and micropore structure, which facilitate the proliferation of bacteria and fungi essential for nutrient absorption by plants, as well as other critical soil processes (Bolan et al., 2023). Furthermore, the results of this study indicated a significant increase in plant yield corresponding to higher doses of biochar. However, it is important to note that the incorporation of excessively high doses of biochar may not necessarily lead to a proportional increase in plant yield (Lehmann et al., 2011; Saletnik et al., 2018).

The community structure and activities of soil microorganisms can be influenced both directly and indirectly by biochar's ability to enhance soil fertility, increase carbon storage, and stabilize and transform contaminants within the soil (Huang et al., 2023). Specifically, the essential characteristics of biomass, including pH, nutrient content, surface area, and pore structure, create favorable conditions for the diversity and proliferation of bacteria and fungi (Chauhan et al., 2023). This microbial activity, in turn, facilitates plant nutrient uptake from the soil (Rashid et al., 2016). Consequently, it is anticipated that as the incorporation of biochar increases plant yield, the activities of microbial and nematode communities will also improve. The findings indicated that the application of biochar resulted in a reduction of migratory ectoparasites and herbivore populations, while simultaneously enhancing the yield of *M.* × *giganteus* in turn.

Conclusions

This study examines the interrelationships between soil amendments and the structure and function of nematode communities within agricultural ecosystems, specifically focusing on the marginal land bioenergy crop *M. x giganteus*. The comprehensive findings not only reaffirm the role of biochar in mitigating plant-parasitic nematodes but also illuminate its beneficial effects on soil nematode communities, microbial diversity, and, ultimately, crop productivity. The recognition that the efficacy of biochar and other organic amendments is dose-dependent and varies according to the specific nematode or microbial community with which it interacts introduces a layer of complexity to the application of these materials in agricultural practices. Notably, the dose-dependent positive effects of biochar on nematode community structure, microbial activity, and the biomass harvested from *M. x giganteus* underscore the potential of customized amendment strategies to enhance agricultural productivity and soil health.

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