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CARBON-MEDIATED RESPONSES OF SOIL NEMATODE COMMUNITIES TO MISCANTHUS-DERIVED BIOCHAR IN A CONTROLLED SWEET CORN POT EXPERIMENT

Tatyana STEFANOVSKA[1]*, Andriy HERTS[2], Oleksandr KONONCHUK[2], Anastasiia HUSIEVA[1], Viktor MARKIV[2], Andrzej SKWIERCZ[3], Olexander ZHUKOV[4], Valentina PIDLISNYUK[5]

[1]Department of Entomology, Integrated Pest Management and Plant Quarantine, National University of Life and Environmental Sciences of Ukraine, Kyiv, 03041, Ukraine
[2]Ternopil Volodymyr Hnatiuk National Pedagogical University, Ternopil, 46027, Ukraine
[3]Department of Plant Protection, The National Institute of Horticultural Research, 96-100 Skierniewice, Poland
[4]Department of Botany and Horticulture, Bogdan Khmelnytskyi Melitopol State Pedagogical University, 72318 Melitopol, Ukraine
[5]Department of Environmental Chemistry and Technology, Jan Evangelista Purkyně University in Ústí nad Labem, 400 96 Ústí nad Labem, Czech Republic

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ABSTRACT

The purpose of this study was to evaluate early chemical and biological responses of soil to miscanthus-derived biochar in a controlled pot system with sweet corn. A short-term pot experiment was conducted using natural soil amended with miscanthus-derived biochar at different rates. Sweet corn was grown under controlled conditions, and soil samples were collected at the start of the experiment and after 60 days in both the with and without plants treatments. Biochar application increased total organic carbon content and modified several soil chemical properties, including nutrient availability, oxidation-reduction potential, and electrical conductivity. Total nematode abundance increased by up to 90% in biochar-amended soils compared with the control, with the strongest response observed in bacterivorous nematodes, indicating stimulation of bacterial energy channels. Multivariate analysis confirmed that soil carbon status and nitrogen availability were the main factors structuring nematode community composition. Overall, these findings show that short-term biochar amendment primarily enhances bacterial-driven soil processes, supporting the use of nematode functional indicators for early assessment of soil response in horticultural systems.

Key words: biochar, carbon stabilization, functional indices, bacterial energy channel, soil nematofauna

INTRODUCTION

Biochar is gaining increasing attention as a soil amendment in horticultural systems due to its potential to modify soil physicochemical properties and influence nutrient dynamics under controlled conditions. Biochar, produced by biomass pyrolysis, is characterized by high carbon stability and a reactive surface that can affect soil pH, oxidation-reduction potential (redox), nutrient availability, and electrical conductivity (Lehmann & Joseph 2015). Numerous studies have demonstrated that biochar addition alters soil chemical and biochemical properties, with important implications for nutrient retention and overall soil function in a wide range of cropping systems (Lehmann et al. 2011). In sweet corn-based systems, biochar has been shown to improve soil physicochemical conditions while simultaneously influencing soil biological activity, highlighting its importance in crop production (Wijitkosum et al. 2025).

*Corresponding author
e-mail: tstefanovska@nubip.edu.ua

At the same time, perennial bioenergy crops such as *Miscanthus* × *giganteus* exhibit a strong response to soil conditions and management practices, particularly under stress or contamination (Pidlisnyuk et al. 2022), further emphasizing the role of soil amendments in shaping plant–soil interactions. Short-term pot experiments are widely used to assess these effects because they allow for precise control of the rate of amendments and environmental variables. However, the rapid agrochemical changes observed in such systems are often difficult to interpret without complementary biological indicators.

Beyond its effects on nutrient availability, biochar is increasingly recognized as a driver of changes in soil carbon pools, both in total and labile carbon fractions. Its persistence in soil and ability to influence carbon stabilization make biochar a key component of soil carbon management strategies (Lehmann & Joseph 2015; Lehmann & Kleber 2015). The form and accessibility of soil carbon play a central role in regulating biological processes in soil, as carbon provides a primary energy source for microorganisms and higher trophic levels in the soil food web. Conceptual frameworks of soil organic matter dynamics emphasize that shifts in carbon stabilization and availability, rather than changes in bulk nutrient concentrations alone, can strongly influence soil functioning and biotic interactions (Lehmann & Joseph 2015; Lehmann & Kleber 2015). Under controlled experimental conditions, soil fauna responses are therefore expected to reflect changes in microbial activity and carbon-mediated resource flow pathways.

Nematodes are among the most widely used biological indicators of soil condition due to their high abundance, functional diversity, and sensitivity to environmental disturbances (Bongers & Ferris 1999; Neher 2001; Sánchez-Moreno & Ferris 2018). As integral components of the soil food web, nematodes respond indirectly to changes in soil chemistry, nutrient availability, and microbial dynamics, making them particularly useful for diagnosing biologically mediated responses to soil amendments. Previous research indicates that biochar can influence soil biota through its effects on microbial communities, habitat structure, and resource availability (Lehmann et al. 2011; Huang et al. 2023). Nevertheless, studies directly assessing nematode responses to biochar remain limited and often report inconsistent outcomes. A previous field study by the authors of this paper also showed early changes in nematode communities after the introduction of organic amendments (Stefanovska et al. 2022). While some experiments have shown little or no effect on overall nematode abundance or diversity (Pressler et al. 2017; Soong et al. 2017), others have documented shifts in trophic structure and functional composition following biochar application (Zhang et al. 2013; Cole et al. 2021). These contrasting findings suggest that nematode responses to biochar are highly context-dependent and closely linked to biochar-induced changes in soil chemical and carbon-related properties.

In horticultural pot systems using biologically active soils, integrated assessments combining soil agrochemical parameters and nematode-based indicators remain rare, particularly in short-term experiments. Such combined approaches are essential for better interpretation of biochar-induced soil changes under controlled conditions and for identifying potential mechanisms underlying biological responses. Therefore, this study aimed to evaluate the effects of short-term miscanthus-derived biochar application at different rates on soil agrochemical properties and nematode-based soil response indicators in a pot-grown system of sweet corn (*Zea mays* L. ssp. *saccharata*).

MATERIALS AND METHODS

Experimental design and crop establishment

The experiment was conducted twice, in two independent runs. Biochar application rates were set to correspond to field equivalents of 1, 3, and 5 $t \cdot ha^{-1}$ (three separate treatments) and converted to appropriate amounts per pot based on soil mass.

The experiment was designed as a short-term, controlled pot study focusing on early soil and biological responses during the initial growth stages (BBCH 11–17) of sweet corn rather than on seasonal or yield outcomes. The pot experiment was conducted under controlled conditions to evaluate the effects of miscanthus-derived biochar on soil agrochemical properties and nematode-based biological indicators. Sweet corn (*Zea mays* L. ssp. *saccharata*), a Spirit F1 hybrid, was used as a model horticultural crop to establish an active rhizosphere environment.

The soil used in the experiment was collected from the research field of Ternopil Volodymyr Hnatiuk National Pedagogical University, Ukraine (49.5418397 N, 25.568175 E). The soil is classified as Chernozem (Phaeozem). Samples were taken from the topsoil of a previously poplar-growing site. Before use, the soil was air-dried, homogenized, and sieved (<2 mm) to ensure uniformity within the individual experimental units. Each treatment was replicated four times (n = 4).

To distinguish the effects of biochar application from plant presence and experiment duration, soil agrochemical properties and nematode communities were assessed at three experimental times.

1. Start phase – assessment immediately after mixing the soil with biochar and before sowing to capture the initial effect of biochar incorporation on soil properties and nematode assemblages.
2. Finish phase with plants – assessment at the end of the experiment (60 days), after sweet corn cultivation, to assess the combined effects of biochar and plants.
3. Finish phase without plants – assessment at the same final stage (60 days) but using parallel pots without plants to distinguish between plant-mediated effects and biochar-induced soil changes.

Sweet corn seeds were sown directly into designated pots after sampling during the start phase. The experiment was conducted in a controlled growth room throughout the 60-day experimental period. Pots containing plants were repositioned every two days within the experimental setup, both within and between rows, to minimize the influence of positional conditions. Plant management included maintaining the room temperature at 20–25 °C, ventilation, artificial lighting at an intensity of 450 µmol photons per m^2 per s (GrowBoard 3.0 100 W, Samsung LM281B+) for 14 h per day, and irrigation to maintain soil moisture at 60%. No additional fertilizers were applied to isolate the effect of biochar on soil chemical and biological indicators.

Biochar was applied at three rates (1 $t \cdot ha^{-1}$, 3 $t \cdot ha^{-1}$, and 5 $t \cdot ha^{-1}$), and within a single rate there were two treatment variants (e.g., D1 and D4) corresponding to different experimental runs with biochar from separate production batches of the same miscanthus feedstock and pyrolysis protocol. There were seven groups as follows: C – unmodified control (0 $t \cdot ha^{-1}$); D1 and D4 – biochar at 1 $t \cdot ha^{-1}$; D2 and D5 – biochar at 3 $t \cdot ha^{-1}$; D3 and D6 – biochar at 5 $t \cdot ha^{-1}$. This design allowed separation of dose-dependent effects from phase-specific responses.

Biochar production and characterization

Miscanthus-derived biochar was produced from pelletized *Miscanthus* × *giganteus* biomass by oxidative pyrolysis under oxygen-limited conditions. The pelletized feedstock consisted of conditioned miscanthus biomass harvested before reaching commercial maturity. Pyrolysis was carried out in an open-top reactor with limited air access (Farmer), developed at the Institute of Renewable Energy of the National Academy of Sciences of Ukraine. The design and operation of the reactor have been previously described by Pidlisnyuk et al. (2025a).

The initial mass of biomass loaded into the reactor was 41.25 kg. Pyrolysis was conducted at 600 °C for 240 min, yielding 9.1 kg of biochar, corresponding to a gravimetric efficiency of approximately 22%. During the process, hot gaseous products were flared to minimize atmospheric emissions.

The physicochemical properties of the biochar obtained from miscanthus were determined before the pot experiment and are presented in Table 1 to document the material characteristics and ensure the reproducibility of the experiment.

Table 1. Physicochemical properties of miscanthus-derived biochar used in the experiment

Property	Measured value	Analytical method (standard)
Specific surface area (m^2 per g)	187	ASTM D6556
Water-accessible pore volume (cm^3 per g)	0.55	ASTM D1513
Moisture content (%)	24.9	ASTM D1509
Volatile matter (%)	8.2	ASTM D1620
Fixed carbon (%)	82.5	ASTM D1762-84
Ash content (%)	9.3	ASTM D1506
Gross calorific value (MJ per kg)	28.1	ASTM D2015

Soil agrochemical analyses

Soil agrochemical parameters were determined using standardized analytical procedures with minor modifications (Pidlisnyuk et al. 2025b). Soil pH (H_2O) was measured according to ISO 10390:2021. Total organic carbon (C_{org}) was determined by the Tyurin method according to DSTU 4289:2004. Nitrate nitrogen (NO_3^-) concentrations were determined according to DSTU 4725:2007. Mobile phosphorus (P) was analyzed by the Chirikov method according to DSTU 4115:2002. Mobile K was measured according to DSTU 4725:2007. Mobile Ca and Na were measured using LAQUAtwin Ca-11 and LAQUAtwin Na-11 meters (Horiba, Japan), respectively. Soil electrical conductivity (EC) was determined using an aqueous extract prepared by mixing soil with deionized water at a ratio of 1 : 5 (w/v). Measurements were performed using a LAQUAtwin B-771 device (Horiba, Japan). Redox in the same extract was measured using a LAQUAtwin ORP-11 meter (Horiba, Japan).

Permanganate oxidizable carbon (POXC) was determined according to an adapted protocol described previously (Weil et al. 2003; Culman et al. 2014).

Nematode extraction and identification

The modified Baermann technique, based on the active migration of motile nematodes from soil into water, was used (Whitehead & Hemming 1965). Fresh soil samples were evenly distributed in a thin layer (approximately 2–3 mm) on a cotton-wool milk filter supported by a plastic sieve or wire mesh with a minimum aperture size of 250 µm. The filter support was placed in a shallow dish. Distilled water was gently added along the side of the dish until the lower surface of the filter just contacted the water, avoiding complete immersion of the soil. Samples were incubated at room temperature for 24–72 h, allowing nematodes to migrate through the filter material and accumulate in the surrounding water. The nematode suspension was then collected by carefully decanting the liquid through a 20–25 µm sieve (Southey 1986). The recovered suspension was transferred to glass beakers for further processing. Extracted nematodes were heat-killed and fixed in TAF solution (formalin and triethanolamine in distilled water). Fixed specimens were gradually transferred to anhydrous glycerol using a slow evaporation method involving successive ethanol–glycerol mixtures (Seinhorst 1959; De Grisse 1969). Permanent slides were prepared in pure glycerol, and samples were placed in a silica gel desiccator to ensure complete removal of residual moisture.

For each sample, up to 100 individuals were examined under a compound light microscope (Zeiss Jena A-Scope). Nematodes were identified to genus level based on morphological and morphometric characteristics using standard taxonomic keys for free-living and plant-parasitic nematodes (Bongers 1988; Siddiqi 2000; Andrássy 2005).

Assessment of nematode community structure and functional indices

Nematode community structure was assessed based on total nematode abundance and the relative abundance of individual taxa. Identified nematodes were assigned to trophic groups according to Yeates et al. (1993) and classified along the colonizer–persister (c–p) scale, which reflects life-history strategies and resource availability. The maturity index (MI) for free-living nematodes and the plant parasite index (PPI) were calculated using genus-specific c–p values weighted by their proportional contribution to the total nematode community (Bongers 1990).

Soil food-web condition and functional characteristics were further evaluated using enrichment, basal, structure, and channel indices as proposed by Ferris et al. (2001). Relationships between enrichment and structure were explored using EI-SI scatter plots. All ecological indices were calculated using the Nematode Indicator Joint Analysis framework (Sieriebriennikov et al. 2014).

Statistical analysis

Because both runs followed an identical protocol and contributed to the same treatment structure, their data were pooled into a single dataset for analysis. Thus, each Phase × Variant combination was represented by four biological replicates in the final analysis.

For statistical analysis, the experimental unit was a single pot. Therefore, replication in both MANOVA and subsequent univariate ANOVAs relied on independent pots. The experiment was conducted in two independent runs with the same design. Because both runs followed an identical protocol and contributed to the same treatment structure, their data were combined into a single dataset for analysis. The effects of Phase, Variant, and their interaction were tested using MANOVA, with overall multivariate significance assessed using Pillai's trace. Subsequent fixed-effects ANOVAs were then performed for the individual response variables using the same factorial structure. Overall model significance was assessed by comparing the fitted model with the residual variance. The proportion of variance explained (R^2) was calculated for each response variable to assess the explanatory power of the model.

Following MANOVA, fixed-effects univariate ANOVA models were derived for individual response variables using the procedure to identify the relative contributions of Phase, Variant, and Phase × Variant. F-values and associated p-values were used to assess statistical significance. Where appropriate, interaction effects were interpreted based on nonparallel response patterns across treatment levels.

Nematode community structure was further explored using redundancy analysis (RDA) to examine relationships between nematode genus composition and soil agrochemical variables. RDA models were fitted with soil chemical parameters as constraining variables. The significance of the constrained ordination and individual axes was evaluated using permutation tests (n = 999). Permutation tests were also applied to assess the independent contribution of individual soil variables to nematode community composition.

All statistical tests were performed using R software (R Core Team 2024). Statistical significance was accepted at $p < 0.05$ unless otherwise stated. Graphical outputs display mean values with error bars to visualize treatment, phase, and interaction effects.

RESULTS

Soil agrochemical responses to biochar application

Overall, soil agrochemical properties varied significantly across experimental treatments and phases. The total model was statistically significant for all agrochemical indicators ($p < 0.01$, predominantly $p < 0.001$), with high explanatory power across variables ($R^2 = 0.597–0.979$; Table 2). Treatment variant (Variant), experimental phase (Phase), and their interaction contributed significantly to the observed variation, although the magnitude of response differed among indicators.

Potassium, nitrate, and redox

Potassium (K), nitrate (NO_3^-), and redox exhibited the strongest and most systematic responses to biochar application (Fig. 1).

Table 2. Total model tests (Model vs. Residuals) for each response fitted within MANOVA

Response	F-ratio	P-level	R^2
C_{org}	5.332	<0.001	0.858
POXC	1.882	0.005	0.597
P_2O_5	2.899	<0.001	0.738
pH	2.897	<0.001	0.738
Redox	6.441	<0.001	0.882
NO_3	5.231	<0.001	0.855
K	35.927	<0.001	0.979
Ca	3.533	<0.001	0.785
Na	6.647	<0.001	0.886
Cond	3.353	<0.001	0.774

C_{org} – total organic carbon, POXC – permanganate oxidizable carbon, Cond – electrical conductivity (EC); Predictors: Phase, Variant, and Phase × Variant; N = 84; DF model = 83, DF residuals = 63

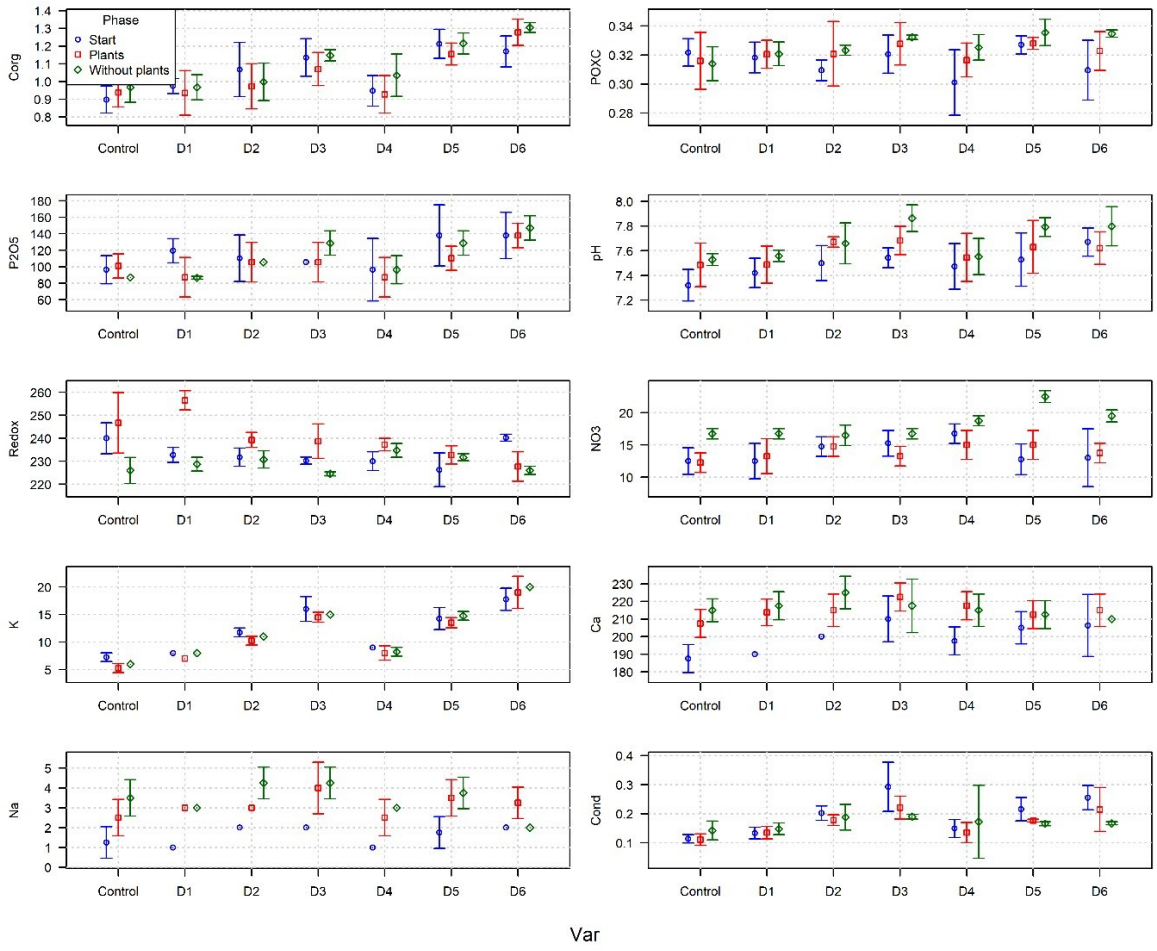

Figure 1. Agrochemical properties of soil across the entire treatment gradient
C_{org} – total organic carbon, POXC – permanganate oxidizable carbon, Cond – electrical conductivity (EC); Means for each Phase × Variant combination with error bars represent measurement variability across the experimental phases (Start, Plants, Without plants) in the control and D1–D6; Nonparallel phase-specific trajectories indicate interaction patterns, and overall differences between phases or variants correspond to main effects according to the fixed-effects ANOVA

Across the treatment gradient, potassium concentrations gradually increased with increasing biochar rate. In the start phase, K values in the control ranged from approximately 236–245 mg·kg^{-1}, whereas biochar-amended treatments showed altered concentrations, reaching 229–241 mg·kg^{-1} across D1–D6, with the highest and most consistent values observed in D3 and D6. During the finish phase with plants, K concentrations increased across all treatments, with control values of approximately 240–257 mg·kg^{-1} and biochar-amended variants reaching 232–260 mg·kg^{-1}. In the finish phase without plants, K concentrations remained elevated but more variable (224–236 mg·kg^{-1} in the control versus 225–233 mg·kg^{-1} in biochar treatments). Across phases, high-rate biochar application resulted in K levels approximately 10–20% higher than the control, consistent with the strong treatment effect and significant Phase × Variant interaction (Table 3).

Nitrate concentrations showed a clear dose-dependent response to biochar application (Fig. 1). In the start phase, NO_3^- concentrations in the control were low (7–8 mg·kg^{-1}) and gradually increased across biochar treatments to 12–18 mg·kg^{-1}, with the highest values in D3 and D6. During the finish phase with plants, NO_3^- concentrations ranged from 5–6 mg·kg^{-1} in the control to 10–21 mg·kg^{-1} in biochar-amended variants, while in the finish phase without plants they remained elevated (6–8 mg·kg^{-1} in the control versus 11–20 mg·kg^{-1} across biochar treatments). Overall, high-rate biochar treatments exhibited NO_3^- concentrations approximately 1.5–3 times higher than the control, with phase-dependent variation in effect magnitude (Table 3).

Table 3. Fixed-effects ANOVA results derived from MANOVA for soil agrochemical properties

Term	Phase	Variant	Phase × Variant
df	2	6	12
C_{org}	5.8*	57.1*	2.1*
POXC	14.2*	6.1*	2.3*
P_2O_5	4.3*	22.8*	2.7*
pH	30.8*	16.0*	1.7 ns
Redox	83.6*	13.8*	18.5*
NO_3	114.9*	10.6*	6.5*
K	13.0*	475.5*	3.3*
Ca	73.2*	6.6*	3.7*
Na	161.2*	16.0*	5.9*
Cond	8.8*	25.1*	3.9*

* indicates $p < 0.05$, ns – not significant; C_{org} – total organic carbon, POXC – permanganate oxidizable carbon, Cond – electrical conductivity (EC); Effects of Phase, Variant, and Phase × Variant (residual df = 63); Values are F-statistics rounded to one decimal place

Redox displayed pronounced phase-dependent shifts and strong treatment-related structuring (Fig. 1). In the start phase, redox values in the control ranged from approximately 87–106 mV, whereas biochar-amended treatments spanned a broader range (105–162 mV), with higher values generally associated with intermediate and high biochar rates. During both finish phases, redox increased across treatments, with biochar-amended variants consistently exceeding the control. Non-parallel phase-specific trajectories across the treatment gradient reflect a strong Phase × Variant interaction (Table 3).

Organic carbon fractions (C_{org} and POXC)

Total organic carbon (C_{org}) showed a clear dose-dependent response to biochar application in all experimental phases (Fig. 1). In the start phase, C_{org} values in the control reached approximately 0.86–1.02%, whereas in the biochar treatments, C_{org} exhibited progressively higher levels, reaching 1.08–1.23% across D1–D6. The highest and most consistent C_{org} levels were observed in D3 and D6, corresponding to an increase of approximately 20–45% compared to the control. In both finish phases, overall C_{org} concentrations increased compared to the start phase. At the same time, a dose-dependent pattern was maintained, resulting in consistently higher C_{org} levels in the high-biochar treatments.

In contrast, permanganate oxidizable carbon (POXC) displayed greater variability across treatment variants and phases (Fig. 1). In the start phase, POXC values in the control were approximately 0.30–0.33, while biochar-amended treatments spanned a similar but broader range (0.29–0.33), with no consistent monotonic increase across all doses. During the finish phases, POXC values extended up to 0.33–0.34 at higher application rates. Although overall model effects for POXC were statistically significant (Table 2), changes across the treatment gradient were smaller and less systematic than for C_{org}, indicating comparatively higher intrinsic variability of the labile carbon fraction.

Phosphorus, base cations, electrical conductivity, and pH

Available phosphorus (P_2O_5), base cations (Ca and Na), electrical conductivity (Cond), pH, and H_2O also responded significantly to the experimental treatments (Table 2; Fig. 1). Across phases, biochar-amended treatments generally exhibited higher P_2O_5 concentrations than the control, with stronger responses at medium and high application rates. Calcium and sodium concentrations showed pronounced differences, and Na exhibited one of the strongest phase effects. Electrical conductivity increased with biochar application rate in all phases, although with different magnitudes. Soil pH remained slightly alkaline, increasing slightly with biochar application, broadly in parallel across phases, (nonsignificant Phase × Variant interaction).

Nematode community responses to experimental factors

Nematode community composition was characterized by low taxonomic richness, with 15 genera detected across all treatments. This limited diversity reflects the short duration and limited conditions of the pot experiment, which favor early succession and taxon opportunism. Overall, multivariate analysis demonstrated that nematode community indices, trophic traces, and aggregate descriptors responded significantly to Phase, Variant, and their interaction (all $p \leq 0.027$; Table 4). The explanatory power of the model varied across responses (R^2 = 0.524–0.901), indicating varying sensitivity of nematode indices to experimental design.

Overall model tests (Model vs. Residuals) for nematode community indices, trophic traces, total abundance, and total biomass were fitted with a MANOVA (predictors: Phase, Variant, and Phase × Variant; N = 84, model df = 83, residual df = 63).

Community indices and trophic traces

All nematode community indices (Maturity, Channel, Basal, and Structure indices) exhibited significant overall model effects (Table 4), while fixed-effects ANOVA revealed significant contributions of Phase, Variant, and their interaction (Table 5). Treatment Variant significantly affected all indices, while strong Phase × Variant interactions indicated phase-dependent treatment responses.

Trophic trace metrics displayed contrasting response patterns (Fig. 2). The bacterivore trace showed the strongest and most structured response, with highly significant effects of Phase, Variant, and their interaction (R^2 = 0.872) (Table 5). In contrast, fungivore and predator traces were dominated by phase effects, with largely additive responses across treatment variants, while the omnivore trace exhibited an intermediate pattern with a significant Phase × Variant interaction but no significant main effect of Variant alone.

Table 4. Summary of fixed-effects ANOVA results per response derived from MANOVA (summary.aov) for Phase, Variant, and their interaction

Response	F-ratio	P-level	R^2
Maturity Index	2.402	<0.001	0.684
Channel Index	2.092	0.001	0.637
Basal Index	2.063	0.002	0.632
Structure Index	1.790	0.008	0.576
Fungivore trace	1.768	0.009	0.571
Bacterivore trace	5.917	<0.001	0.872
Predator trace	1.768	0.009	0.571
Omnivore trace	1.913	0.004	0.603
Total number	7.681	<0.001	0.901
Total biomass	1.594	0.027	0.524

Table 5. Fixed-effects ANOVA results derived from MANOVA for nematode community indices, trophic traces, total abundance, and total biomass

Term	Phase	Variant	Phase × Variant
df	2	6	12
Maturity Index	4.2*	8.4*	6.5*
Channel Index	1.1 ns	4.6*	6.7*
Basal Index	1.1 ns	4.4*	6.6*
Structure Index	0.6 ns	3.7*	5.2*
Fungivore trace	19.1*	4.4*	1.6 ns
Bacterivore trace	98.3*	12.6*	13.0*
Predator trace	19.1*	4.4*	1.6 ns
Omnivore trace	28.3*	1.2 ns	2.7*
Total number	191.2*	13.2*	9.4*
Total biomass	4.1*	6.3*	1.9*

* indicates $p < 0.05$, ns – not significant; Values are F-statistics rounded to one decimal place; Columns represent model terms and rows represent response variables; Degrees of freedom were 2 for Phase, 6 for Variant, and 12 for Phase × Variant

Nematode abundance and bacterivore trace

Total nematode abundance exhibited the strongest quantitative response among all nematode-related indicators (Fig. 2). In the start phase, the lowest abundances were observed in the control. In contrast, abundances in the biochar treatments gradually increased across the gradient, with the highest values in D3 and D6. In the finish phase with plants, total nematode abundance increased in all treatments, with biochar treatments exceeding the control by approximately 30–70%, depending on the dose. In the finish phase without plants, abundances remained higher than in the start phase, and high-rate biochar treatments maintained values approximately 40–90% higher than the control. These pronounced and phase-dependent differences are consistent with the very high explanatory power of the model for total number (R^2 = 0.901; Table 5). The bacterivore trace showed an even more structured response to biochar application (Fig. 2).

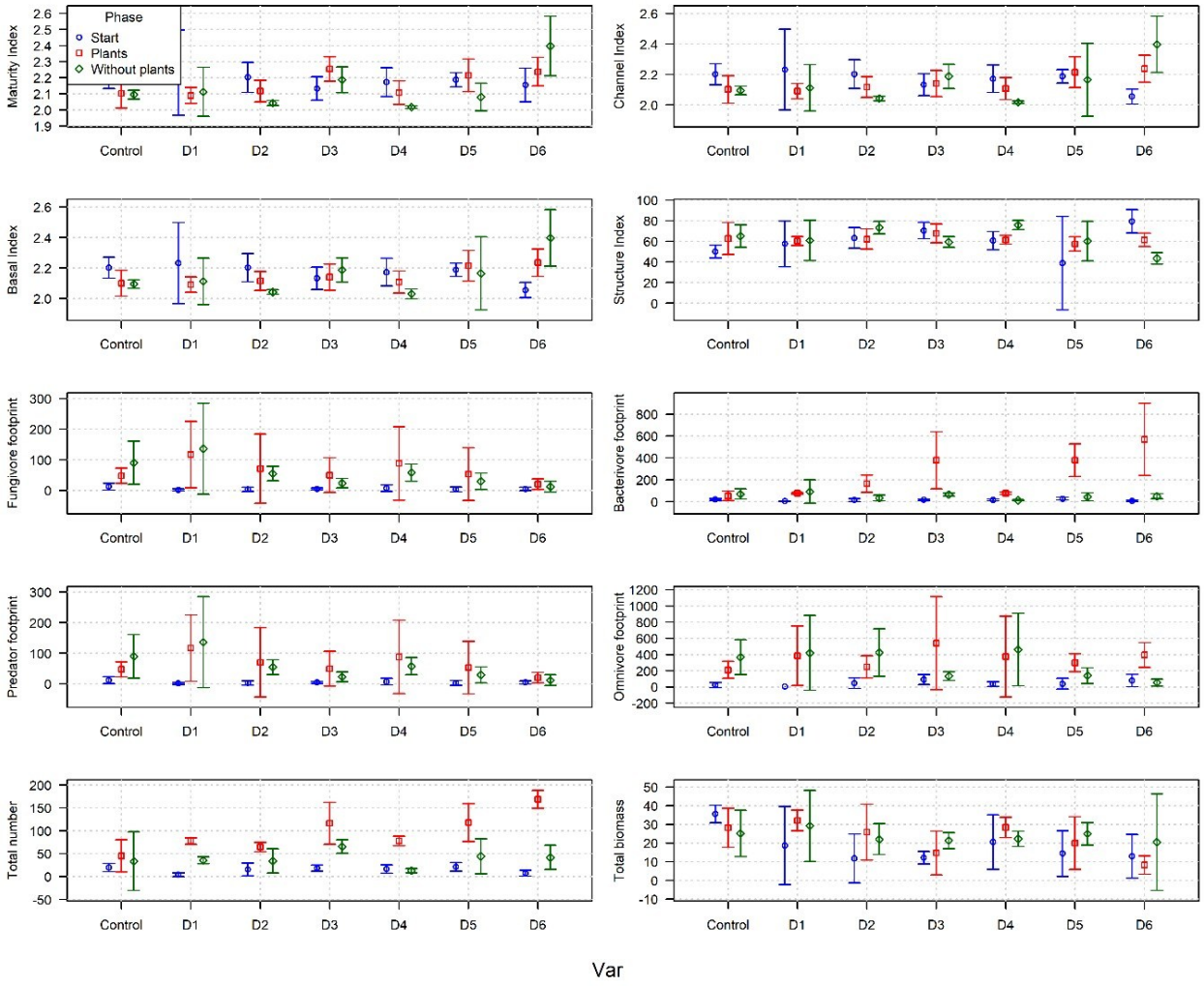

Figure 2. Nematode community indices, trophic traces, total abundance, and total biomass across the entire treatment gradient

Means for each Phase × Variant combination with error bars represent measurement variability across the experimental phases (Start, Plants, Without plants) in the control and D1–D6; Visual differences between trajectories for individual phases reflect the response structure associated with phase, variant, and their interaction; Statistical significance according to the fixed-effects ANOVA

In the start phase, bacterivore trace values were lowest in the control and gradually increased across biochar treatments. During the finish phase with plants at a high biochar dose, bacterivore trace values were approximately 2-3 times higher than in the control, indicating a strong amplification of the bacterial energy channel. In the finish phase without plants, bacterivore trace values remained elevated, although variability between treatments increased slightly. The strength and consistency of these patterns are reflected in the high explanatory power of the model and the highly significant effects of Phase, Variant, and Phase × Variant (Table 5).

Associations between soil agrochemical properties and nematode communities

Redundancy analysis revealed strong and statistically supported associations between soil agrochemical properties and nematode community composition (Fig. 3). The constrained model explained a substantial proportion of the variance in nematode genus composition (adjusted $R^2 = 0.429$), and both primary constrained axes were highly significant ($p = 0.001$).

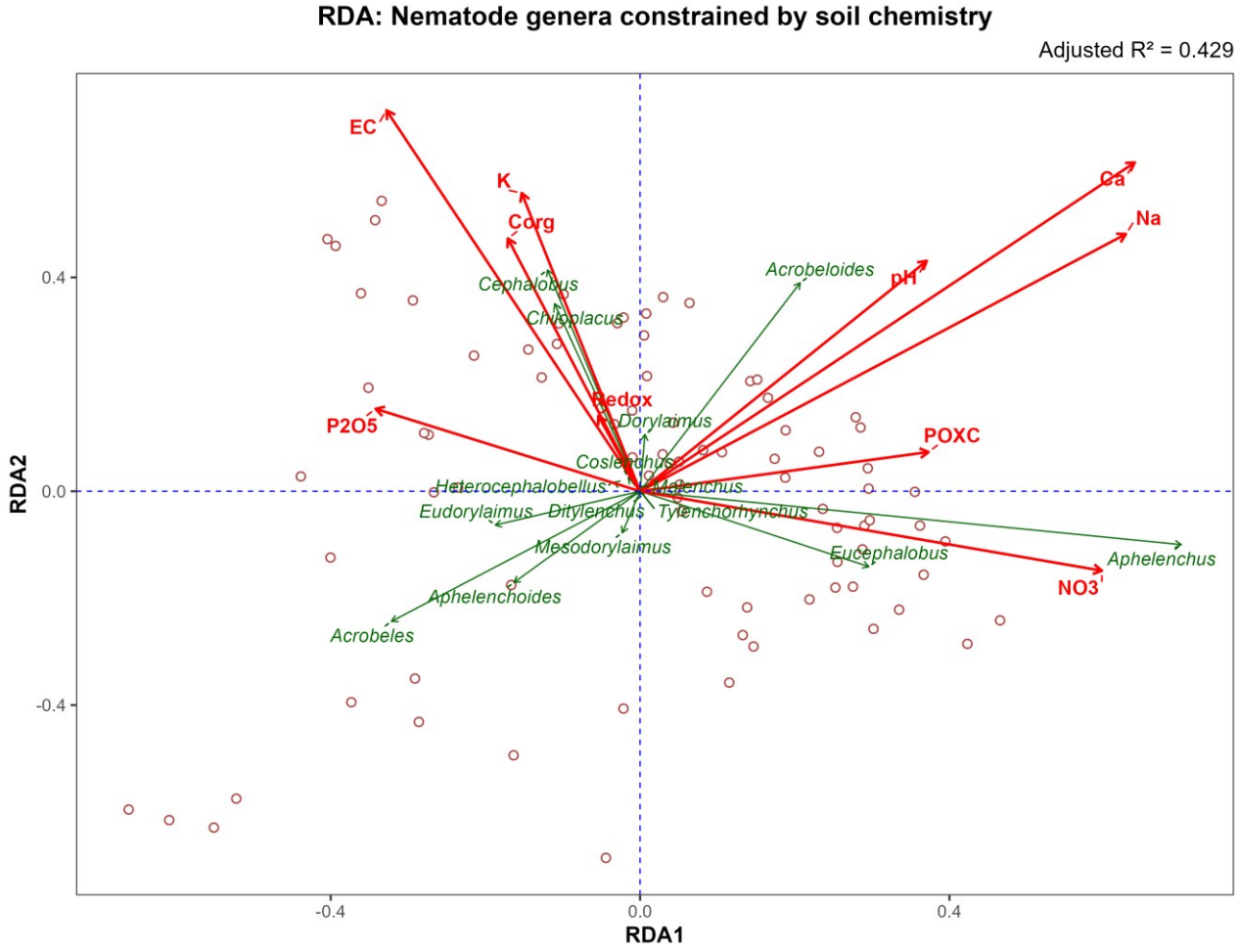

Figure 3. Redundancy analysis (RDA) ordination of nematode genera based on soil agrochemical properties

C_{org} – total organic carbon, POXC – permanganate oxidizable carbon, EC – electrical conductivity (Cond); Points represent sampling sites, text labels denote nematode genera, arrows indicate soil chemical variables, and their length and orientation reflect the strength and direction of correlations between environmental variables and ordination axes; The constrained model explains 42.9% of the variation in nematode genus composition (adjusted $R^2 = 0.429$)

Among the soil variables analyzed, total organic carbon (C_{org}) emerged as the strongest individual predictor of nematode community structure ($F = 13.22$, $p = 0.001$), followed by nitrate (NO_3^-) ($F = 9.88$, $p = 0.001$). Permanganate oxidizable carbon (POXC) also contributed significantly to the constrained ordination ($F = 7.52$, $p = 0.001$), indicating that both total and labile carbon fractions were closely associated with variation in nematode assemblages. Calcium, electrical conductivity, redox, soil pH, and available phosphorus also showed significant associations (all $p = 0.001$), whereas potassium and sodium contributed more weakly but remained statistically significant predictors. Ordination also revealed genus-specific relationships with these gradients. *Aphelenchus* and *Eucephalobus* were positioned in the direction of the NO_3^- and POXC vectors, corresponding to higher nitrate availability and labile carbon. *Acrobeloides* was aligned with the pH, Na, and Ca vectors, consistent with more alkaline, cation-enriched conditions. In contrast, *Acrobeles* and *Aphelenchoides* were located on the opposite side of the main carbon- and ion-related gradients, corresponding to relatively lower values of these variables. Genera located closer to the plot center showed weaker or less specific relationships with individual chemical factors. The ordination demonstrates that variation in nematode community composition was structured primarily along gradients of soil carbon and nitrogen-redox-ion conditions.

DISCUSSION

Carbon-mediated regulation of soil conditions by miscanthus-derived biochar

The key novelty of this study lies in demonstrating that short-term soil biological responses to miscanthus-derived biochar in a horticultural pot system are primarily mediated by changes in soil carbon status rather than by direct nutrient enrichment. By integrating agrochemical indicators with nematode-based functional metrics under controlled conditions, biochar was shown to primarily regulate carbon-driven soil processes during early crop establishment.

The present study demonstrates that short-term application of miscanthus-derived biochar induced pronounced and coherent changes in soil agrochemical properties, with soil carbon status emerging as a central structuring factor. Such carbon-centered effects are consistent with the extensive evidence showing that biochar acts primarily as a stable carbon amendment, capable of modifying soil physicochemical conditions beyond the direct nutrient inputs (Sohi et al. 2010; Spokas et al. 2012; Tian et al. 2024; Biswal & Balasubramanian 2025). In the present experiment, total organic carbon (C_{org}) showed a clear, dose-dependent increase across all experimental phases. In contrast, permanganate oxidizable carbon (POXC) showed a more variable response but remained significantly associated with treatment effects. This pattern supports the concept that biochar alters soil functioning predominantly through changes in carbon quantity and accessibility, rather than through isolated shifts in individual nutrients.

The contrasting behavior of total organic carbon (C_{org}) and permanganate oxidizable carbon (POXC) indicates that nematode responses were not driven solely by increases in readily available carbon, but rather by broader shifts in carbon availability and stabilization induced by biochar. This interpretation is consistent with conceptual framework that emphasizes the role of carbon stabilization and accessibility in regulating soil biological processes (Lehmann & Kleber 2015).

The dominant contribution of C_{org} to the multivariate structure of soil properties and nematode assemblages indicates that carbon enrichment from biochar is a major driver of biological responses in this pot system. Increases in C_{org} were accompanied by coordinated shifts in redox and nitrate concentrations, suggesting a coupled carbon-nitrogen-redox control of soil processes under controlled conditions. Similar carbon-driven linkages between biochar application, redox dynamics, and nitrogen availability have been reported in recent meta-analyses and synthesis studies (Bekchanova et al. 2024).

Importantly, carbon-driven agrochemical changes were consistently observed across experimental phases, including during sweet corn growth and in pots without plants, indicating that the early biochar effects on soil carbon pools were not solely mediated by plant activity. This is particularly relevant for horticultural systems, where substrate preparation and early biological conditioning often precede full root development and plant-driven feedback.

Carbon-driven shifts in nematode abundance and bacterial energy channels

Our results demonstrate that short-term biochar amendment rapidly activates bacterial-driven energy channels in soil, with nematode functional indicators providing sensitive early signals of carbon-mediated biological restructuring.

The most pronounced biological responses were observed for total nematode abundance and the bacterivore trace, which both showed strong, dose-dependent increases across the experimental phases. Such patterns are consistent with soil food web theory, which identifies bacterivorous nematodes as rapid responders to changes in carbon availability and microbial activity (Ferris et al. 2001; Neher 2010). These findings are consistent with recent evidence that biochar improves soil biological health (Bolan et al. 2024). In biochar-amended treatments, total nematode abundance increased by approximately 30–90% compared to the control, while bacterivore trace increased 2–3-fold at higher application rates. These responses were strongly associated with increases in total organic carbon (C_{org}) and, to a lesser extent, permanganate oxidizable carbon (POXC), as demonstrated by redundancy analysis. This indicates that nematode responses were driven primarily by carbon-mediated changes in microbial resource availability rather than by direct nutrient effects. This pattern is consistent with observations from agroecosystems, where nematode community responses are closely linked to soil conditions and management practices (Babych et al. 2024). Similar carbon-driven stimulation of bacterivore-dominated energy pathways has been reported in studies of carbon manipulation in agricultural soils (Li et al. 2021).

In contrast, higher trophic levels exhibited limited responses, as predatory and omnivorous nematodes remained scarce in all treatments, indicating constraints in the development of more complex trophic structures. This pattern should be interpreted as a characteristic feature of early successional stages in confined horticultural pot systems rather than as an indication of reduced ecological functionality. Higher trophic levels typically require longer timeframes and more structurally complex habitats to establish stable populations (Bongers 1990; Ferris et al. 2001). The short experimental duration (60 days), combined with the spatial constraints inherent in pot systems, therefore favored rapid responses of lower trophic levels.

The initial soil used in this study originated from a natural site previously dominated by poplar vegetation, likely supporting relatively simple nematode assemblages with low baseline abundance of predatory taxa. Transferring such soils to controlled pot conditions may further constrain the establishment of higher trophic levels, reinforcing the dominance of bacterivorous nematodes during the early stages of biochar-induced soil adjustment.

Feedstock-specific carbon quality as a driver of soil-nematode interactions

The observed nematode responses emphasize that biochar effects on soil biology depend strongly on feedstock-specific carbon quality and stability, not solely on the rate of application. Miscanthus-derived biochar, produced through oxidative pyrolysis at approximately 600 °C, is characterized by a high fixed carbon content, a developed specific surface area, and a balanced spectrum of functional groups, which together favor carbon persistence while simultaneously creating microsites that support microbial colonization and activity (Pidlisnyuk et al. 2022; Premalatha et al. 2023).

Such feedstock-specific carbon properties provide a mechanistic basis for the carbon-driven biological effects observed in this study. Although a substantial fraction of biochar-derived carbon is chemically recalcitrant, its presence can indirectly enhance microbial activity by improving microsite conditions and stabilizing microbial substrates.

As a result, bacterivorous nematodes, which are closely coupled to bacterial biomass and turnover, respond rapidly to biochar-induced changes in soil conditions.

In contrast, predatory and omnivorous nematodes exhibited limited responses across all treatments. This pattern is consistent with trait-based studies showing that functional responses of nematode communities to changes in soil resources may occur without marked shifts in overall taxonomic diversity, particularly under short-term or controlled conditions (Hou et al. 2023). Long-term field experiments suggest that increases in predator abundance typically emerge only as soil food webs mature over extended timescales (Jia & de Goede 2025).

Taken together, these findings indicate that miscanthus-derived biochar acts primarily as a regulator of soil carbon dynamics and microbial habitat quality, with cascading effects on nematode functional structure. The dominance of bacterial-driven energy channels observed here reflects an early-stage, carbon-mediated reorganization of the soil food web rather than a limitation of biochar effectiveness. From a horticultural perspective, such rapid activation of bacterial-mediated processes may be advantageous during crop establishment, as it signals active biological adjustment of amended substrates under controlled cultivation conditions.

The present study has several limitations that define the scope of interpretation. The short-term duration (60 days) captures early-stage responses and does not reflect longer-term soil biological dynamics or carbon stabilization processes. The controlled pot system imposes spatial constraints that may limit the development of more complex soil food webs, particularly higher trophic levels. In addition, homogenized conditions reduce environmental variability compared with field systems. Therefore, extrapolation of these findings to field conditions should be done with caution and requires further validation through long-term field experiments.

CONCLUSIONS

The short-term application of miscanthus-derived biochar induced pronounced changes in soil chemical properties and strongly stimulated soil biological activity, with total organic carbon emerging as the primary driver of nematode community responses. Biochar amendment promoted a rapid increase in total nematode abundance and a clear dominance of bacterivorous nematodes, indicating an early activation of bacterial-mediated energy pathways in the soil food web. The limited presence of predatory and omnivorous nematodes reflects the short duration and confined conditions of the pot experiment rather than a reduced ecological potential of biochar-amended soils.

The contrasting responses of total organic carbon and labile carbon fractions highlight the importance of distinguishing between carbon pools when interpreting biological responses to biochar. However, the absence of direct measurements of total soil carbon dynamics and long-term carbon stabilization processes represents a key limitation of the present study.

Future research should therefore focus on long-term field or semi-field experiments to assess the persistence of biochar-induced changes in soil carbon pools and their cascading effects on higher trophic levels of the soil food web. Integrating detailed carbon fractionation, microbial functional analyses, and trait-based nematode indicators will be essential for understanding how biochar-mediated carbon dynamics influence soil biological stability and productivity in horticultural systems.

Author contribution statement

TS – A – Research concept and design, C – Data analysis and interpretation, D – Writing the article
AHerts – A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation
OK – A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation
AHusieva – B – Collection and/or assembly of data, C – Data analysis and interpretation
VM – B – Collection and/or assembly of data
AS – C – Data analysis and interpretation, E – Critical revision of the article
OZ – C – Data analysis and interpretation, D – Writing the article
VP – E – Critical revision of the article, F – Final approval of the article

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