

# Use of agricultural wastes to reduce toxicity effect of tetracycline on soil nematode community

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**Abstract:** In order to remove soil tetracycline residue and identify the effect of tetracycline on soil nematode community, agricultural waste returning was applied in a maize monocropping field, northeast China. The results showed that plant parasites were the dominant genera in high concentration of soil tetracycline; however, bacterivores were the dominant genera in all organic matter amendments. Maturity index, structure index and enrichment index showed the highest values in biochar and compost mixed amendments and these treatments had the highest tetracycline removal rate and the highest concentration of macro-aggregates, total organic C and available N, followed by biochar separate amendments. Overall, biochar and compost mixed amendments efficiently reduced the risk of soil tetracycline pollution below the threshold, with the characteristics of cheap, improving soil fertility and above all, environmentally friendly.

**Keywords:** antibiotic; manure; microfauna; soil pollution; soil remediation

As one of the emerging contaminants in the soil, antibiotics have been widely applied for animal drugs and feed additives to improve animal health (Kayal & Mandal 2022) and tetracyclines, including tetracycline, chlortetracycline, oxytetracycline and doxycycline, are the most commonly used antibiotics worldwide (Kessler et al. 2020). Driven by economic interests, overuse of tetracyclines is normal, and over 85% of tetracyclines were released in unchanged forms via urine and faeces (Li et al. 2025), resulting in tetracyclines largely accumulating in the soil.

Since tetracyclines are toxic to soil microorganisms, animals, plants, and even humans, long-term use of tetracyclines greatly increases ecological risk.

Soil nematodes are one of the most important metazoa, which are important regulators of organic matter decomposition and nutrient release through their high turnover rates and their interactions with microflora (Ferris et al. 2001) and they have been widely used as bioindicators to assess soil contamination caused by agricultural management as they respond rapidly to changes in the soil environment (Zhong

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et al. 2025). Accumulated soil tetracyclines greatly affected the abundance and biomass of nematodes. For example, Hernando and Bouzat (2025) found that LC50 of tetracycline for *Caenorhabditis elegans* was 52.9 mg/kg (24 h). Clausi et al. (2021) found that the growth rate of entomopathogenic nematodes greatly slowed down after exposure to tetracycline. Thus, we hypothesised that tetracyclines may have a significant disturbance on the soil nematode community.

Agricultural wastes are low-cost and effective soil amendments to decrease the toxicity of tetracyclines that affects the soil nematode community. The mechanisms are explained as follows: (1) the continuous high temperature (55–65 °C) during composting provides a suitable environment for tetracycline-degrading bacterial reproduction, and the removal rates are generally over 85% (Mousavi et al. 2024). (2) Biochar has a porous structure, a large specific surface area, and abundant functional groups such as -COOH and -OH, which effectively immobilise tetracyclines (Al-Wabel et al. 2021). (3) Tetracyclines have three forms, including cationic (pH > 7.2), zwitterionic (pH = 6.0–7.2) and anionic (pH = 2.5–6.0). The cationic form was the most closely combined with soil organic matter through hydroxyl and carbonyl (Zhu et al. 2025), which reached a maximum adsorption capacity of 864.3 mg/g in 3.5 h (Chen et al. 2020). However, Zhou et al. (2023) found that the adsorption capacity decreased with the decrease in soil pH. Therefore, tetracyclines can be desorbed by adjusting soil pH to 5.5–6.0.

Overall, agricultural wastes are efficient soil amendments for soil tetracycline removal. Therefore, the objective of this study was to use compost and biochar combinations to remove soil tetracycline residue and improve soil quality, benefit the soil nematode community structure in the soil food web under maize cultivation.

## MATERIALS AND METHODS

**Site descriptions.** The experiment was carried out on the Lishu Experimental Station (43°56'39.9"N, 124°31'50.3"E), Chinese Academy of Sciences, since 2013. The test soil is classified as Entisols, silty loam in USDA texture system with physicochemical properties of 35.3 % sand, 46.8 % silt, 17.9 % clay, 12.6 g per kg total organic C, 1.22 g/kg total N, 0.96 g/kg total P, 1.33 g/kg total K and pH 6.59.

The field experiment was divided into ten plots (10 × 10 m<sup>2</sup> each) under a maize monocropping

conventional tillage, with four replicates, distributed in a completely randomised block design. Treatments were no addition (CK), tetracycline (T), biochar plus tetracycline (B), compost plus tetracycline (C), 1/2 biochar + 1/2 compost plus tetracycline (I). The tetracycline (ultra-pure, hydrochloric salt, C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>8</sub>) was purchased from Aladdin. The compost was aerobically composted with maize straw and chicken manure. Biochar (< 0.5 mm) was prepared from maize straw and chicken manure under an oxygen-limited furnace at 600 °C for 5 h. Physicochemical properties of each amendment and field management were shown in Tables S1 and S2 in the Electronic Supplementary Material (ESM).

**Soil sampling and analysis.** Soil samples were collected at 0–20 cm at the seedling stage (June 16, 2025), jointing stage (July 22, 2025), booting stage (August 28, 2025) and ripening stage (October 9, 2025). Each soil sample pooled from five soil cores (2.5 cm diameter) was stored in individual plastic bags and transferred to a 4 °C icebox. Soil tetracycline, properties and nematode analyses were described in Table S3 in the ESM. Meaning and calculation of nematode indicators were described in Table S4 in the ESM.

**Statistical analysis.** Nematode data were  $\ln(x + 1)$  transformed prior to statistical analysis. Data were analysed by SPSS statistical software (SPSS Inc., Chicago, IL, USA). Means were compared between treatments and sampling stages by least significant difference (LSD). Difference at  $P < 0.05$  level was considered as statistically significant. Since samples were collected four times from the same plots, stage was used as a repeated measure.

## RESULTS

**Soil property.** Significant treatment effect was for soil moisture, total organic C, available N, and macro-aggregates ( $P < 0.01$ , Table 2). Biochar input significantly increased pH and available N; compost input significantly increased total organic C and total N; mixed input of compost and biochar significantly increased macroaggregates and porosity in comparison with CK. Soil moisture was 36.6% higher in the mixed amendments than in CK, with the order: T < CK < C < B < I (Figure 1). The concentration of each indicator significantly increased with the increase of the additive amount of each amendment. Soil tetracycline concentration varied with the following order: I < B < C < CK < T, and the concentration

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Table 1. Average contribution (%) of different nematode genera to total nematodes in different treatments

Genus	Treatment											Guilds <sup>a</sup>
	CK	T	LC	MC	HC	LB	MB	HB	LI	MI	HI	
Bacterivores	30.37	19.31	36.79	38.33	39.61	38.40	41.39	39.87	37.71	39.80	42.90	
Pelodera*	3.61	0.00	5.93	5.63	5.72	4.27	4.42	6.24	6.18	5.29	6.08	Ba1
Mesorhabditis	2.81	1.17	0.71	1.36	1.74	0.79	3.59	1.64	1.06	1.99	3.66	Ba1
Panagrolaimus	0.00	1.13	1.33	1.26	2.26	2.38	2.50	2.03	0.89	0.00	1.31	Ba1
Monhystera	1.43	2.93	0.69	0.00	3.24	3.22	0.00	2.39	0.00	0.00	0.00	Ba1
Heterocephalobus	1.45	1.03	2.90	1.81	3.83	3.66	4.24	2.78	3.27	3.77	3.32	Ba2
Eucephalobus	1.47	0.00	1.70	2.08	1.34	1.16	0.00	0.70	1.16	1.51	1.03	Ba2
Acrobeloides*	6.61	5.47	6.40	7.83	4.39	1.68	5.08	3.88	2.90	4.41	5.69	Ba2
Chiloplacus	0.45	0.00	1.06	1.34	1.12	1.45	0.00	1.31	1.15	0.00	1.86	Ba2
Plectus	0.99	1.46	0.00	0.00	1.28	0.88	1.08	0.96	1.50	1.47	0.82	Ba2
Chronogaster	0.00	0.00	0.00	0.00	1.49	2.38	0.00	0.86	0.00	0.00	1.21	Ba2
Leptolaimus	1.03	2.29	1.33	1.11	0.00	0.76	0.00	0.00	3.34	0.00	1.30	Ba2
Chromadorita	1.38	0.00	0.00	1.16	0.92	2.23	3.16	0.99	0.86	0.00	1.33	Ba3
Teratocephalus	0.90	1.51	1.08	0.97	1.47	1.74	2.92	1.86	1.38	0.95	1.52	Ba3
Rhabdolaimus	2.03	0.00	1.75	1.69	2.32	1.27	1.86	2.61	2.52	2.12	2.39	Ba3
Aulolaimus	0.00	0.00	0.00	0.00	1.07	0.97	1.12	2.46	1.80	2.47	1.70	Ba3
Paracyatholaimus	1.18	0.54	3.27	3.02	0.45	1.98	2.20	0.82	1.45	1.66	1.34	Ba3
Prismatolaimus*	5.03	1.78	5.10	5.69	4.07	4.49	6.79	5.63	6.17	6.22	5.02	Ba3
Alaimus	0.00	0.00	3.54	1.50	1.61	2.11	0.86	1.13	0.76	2.95	1.34	Ba4
Paramphidelus	0.00	0.00	0.00	1.88	1.29	0.98	1.57	1.58	1.32	4.99	1.98	Ba4
Fungivores	18.72	16.23	21.05	22.91	24.24	22.19	24.56	23.49	20.96	23.44	25.77	
Ditylenchus*	6.51	6.40	3.41	5.34	5.23	6.27	1.17	4.59	1.09	1.16	1.22	Fu2
Aphelenchus*	8.55	3.56	6.39	6.00	6.95	3.18	2.82	4.34	4.31	4.12	5.63	Fu2
Aphelenchoides*	0.00	2.78	5.14	7.31	6.03	4.72	5.36	0.00	3.62	4.20	3.40	Fu2
Dorylaimoides*	0.00	0.00	2.55	1.37	1.60	2.19	7.91	8.71	6.23	7.93	8.47	Fu4
Tylencholaimus*	3.66	3.49	3.56	2.89	4.43	5.83	7.30	5.85	5.71	6.03	7.05	Fu4
Omnivore–carnivores	21.26	20.86	22.38	23.23	24.30	21.68	24.91	22.50	26.21	26.33	24.95	
Mononchus*	2.11	3.83	2.50	2.13	4.13	2.46	6.99	3.29	9.87	7.10	1.23	Ca4
Prodorylaimus	1.43	1.60	0.00	1.13	0.80	1.14	3.03	0.00	3.44	8.42	2.10	Ca5
Mesodorylaimus*	1.67	6.01	1.49	4.11	1.43	1.93	1.33	0.00	3.09	1.86	1.85	Ca5
Thonus*	2.66	2.18	4.37	2.98	5.68	2.89	1.70	6.49	1.66	1.22	2.62	Om4
Epidorylaimus	0.00	0.00	1.58	1.26	0.00	4.29	0.00	2.17	0.00	1.63	3.72	Om4
Dorydorella	3.14	0.00	2.85	2.25	0.00	1.76	1.93	2.06	2.96	1.54	2.67	Om4
Kochinema	1.11	2.43	1.28	1.87	1.17	1.29	2.24	1.75	4.56	3.52	1.78	Om4
Aporcelaimellus	0.99	3.58	2.59	1.41	3.78	2.47	2.57	2.41	0.00	0.00	1.52	Om5
Aporcelaimus*	5.59	1.23	3.65	4.62	6.68	3.45	3.90	2.42	0.00	1.04	6.16	Om5
Discolaimium	2.56	0.00	2.07	1.47	0.63	0.00	1.22	1.91	0.63	0.00	1.30	Om5
Plant parasites	29.65	43.60	19.78	15.53	11.85	17.73	9.14	14.14	15.12	10.43	6.38	
Coslenchus	2.46	2.17	1.98	0.00	2.33	1.39	0.00	0.81	0.94	0.47	2.06	PP2
Boleodors*	5.30	5.12	1.54	2.62	1.24	1.11	1.03	0.95	1.60	1.03	1.65	PP2
Filenchus**	10.10	4.91	6.43	2.50	0.47	1.88	1.84	1.54	3.18	1.08	0.00	PP2
Psilenchus	0.92	0.00	1.81	0.68	0.82	1.14	2.30	1.03	1.02	1.16	1.11	PP2
Paratylenchus	1.10	0.00	0.98	0.00	1.38	2.26	0.00	0.73	0.94	1.21	0.00	PP2

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Table 1 to be continued

Genus	Treatment											Guilds <sup>a</sup>
	CK	T	LC	MC	HC	LB	MB	HB	LI	MI	HI	
Meloidogyne	0.00	4.07	2.88	0.80	0.00	2.51	0.00	0.45	0.00	0.85	0.00	PP3
Rotylenchus	1.54	2.45	0.00	1.83	0.76	0.00	0.00	0.00	0.78	1.19	1.22	PP3
Pratylenchoides*	0.00	8.94	0.00	1.24	0.00	1.29	0.89	1.63	0.00	0.00	0.00	PP3
Criconemoides*	5.32	1.33	3.94	1.77	2.06	3.31	0.97	2.53	1.10	2.36	0.34	PP3
Hemicycliophora**	0.70	10.33	0.00	0.00	1.17	1.08	0.67	0.64	0.52	0.00	0.00	PP3
Trichodorus	0.00	0.00	0.00	1.91	1.06	0.00	0.93	1.72	3.94	0.00	0.00	PP4
Oxydirus	2.21	4.28	0.22	2.18	0.56	1.76	0.51	2.11	1.10	1.08	0.00	PP5

CK – no addition; T – tetracycline; B –, biochar + T; C – compost + T; I – 50% compost + 50% biochar + T; L – 40 t/ha; M – 60 t/ha; H – 80 t/ha; <sup>a</sup>functional guilds of soil nematodes characterized by feeding habits and life-history characters, numbers following the functional guilds indicate the c-p values (Bongers & Bongers 1998; Ferris et al. 2001); \*\*dominant genus, average contribution (%) of different nematode genera to total nematodes is more than 10%; \*subdominant genus, average contribution (%) is over 5%

significantly decreased with the increase of the additive amount of each amendment (Figure 2).

Significant stage effect was for porosity, pH and total N ( $P < 0.05$ , Table 2). Most soil properties exhibited a seasonal fluctuation, with the highest values in the booting stage and the lowest in the seedling stage.

**Nematode composition.** Mixed amendments greatly increased the abundance of total nematodes in comparison with biochar and compost separated

input, and on average 86.9% higher in the I treatment than in CK (Figure 2). *Prismatolaimus* and *Tylencholaimus* in mixed amendments, *Dorylaimoides* in biochar amended treatments, *Pelodera* in compost amended treatments, *Hemicycliophora* in T treatment and *Filenchus* in CK were the dominant genera (Table 1).

The relative abundance of bacterivores was much higher in all organic amendments than in CK

Table 2. Effects of different treatments, sampling stages, and interaction between these variables on soil nematodes and soil properties

ANOVA	Treatment (T)	Stage (S)	T × S	ANOVA	Treatment	Stage	T × S
	P-value				P-value		
TNEM	0.022	ns	ns	EI	< 0.01	ns	ns
BF	< 0.01	ns	0.033	SI	0.044	ns	ns
FF	< 0.01	ns	ns	SM	ns	< 0.01	ns
OC	0.013	< 0.01	ns	PO	ns	ns	< 0.01
PP	ns	0.017	< 0.01	MA	< 0.01	ns	ns
H'	< 0.01	< 0.01	ns	pH	ns	0.022	< 0.01
SR	< 0.01	< 0.01	ns	TOC	0.031	ns	ns
MI	< 0.01	ns	ns	TN	ns	0.028	ns
PPI	ns	ns	< 0.01	AN	< 0.01	ns	ns
BI	< 0.01	< 0.01	ns	ST	< 0.01	ns	< 0.01
CI	0.043	ns	< 0.01				

TNEM – total nematodes; BF – bacterivores; FF – fungivores; OC – omnivore-carnivores; PP – plant parasites; H' – Shannon-Weaver diversity; SR – species richness; MI – maturity index; PPI – plant parasite index; BI – basal index; CI – channel index; EI – enrichment index; SI – structure index; SM – soil moisture; PO – porosity; MA – macro-aggregates; TOC – total organic C; TN – total N; AN – available N; ST – soil tetracycline

(Table 1). The abundance of bacterivores with c-p 3-4 was 39.7% and 52.3% higher in I treatments than in B and C treatments, respectively. The highest abundance of fungivores, omnivores, and

carnivores was represented in the I treatments, followed by the B treatments, and the T treatment showed the lowest abundance (Figure 2). The abundance of plant parasites oppositely showed

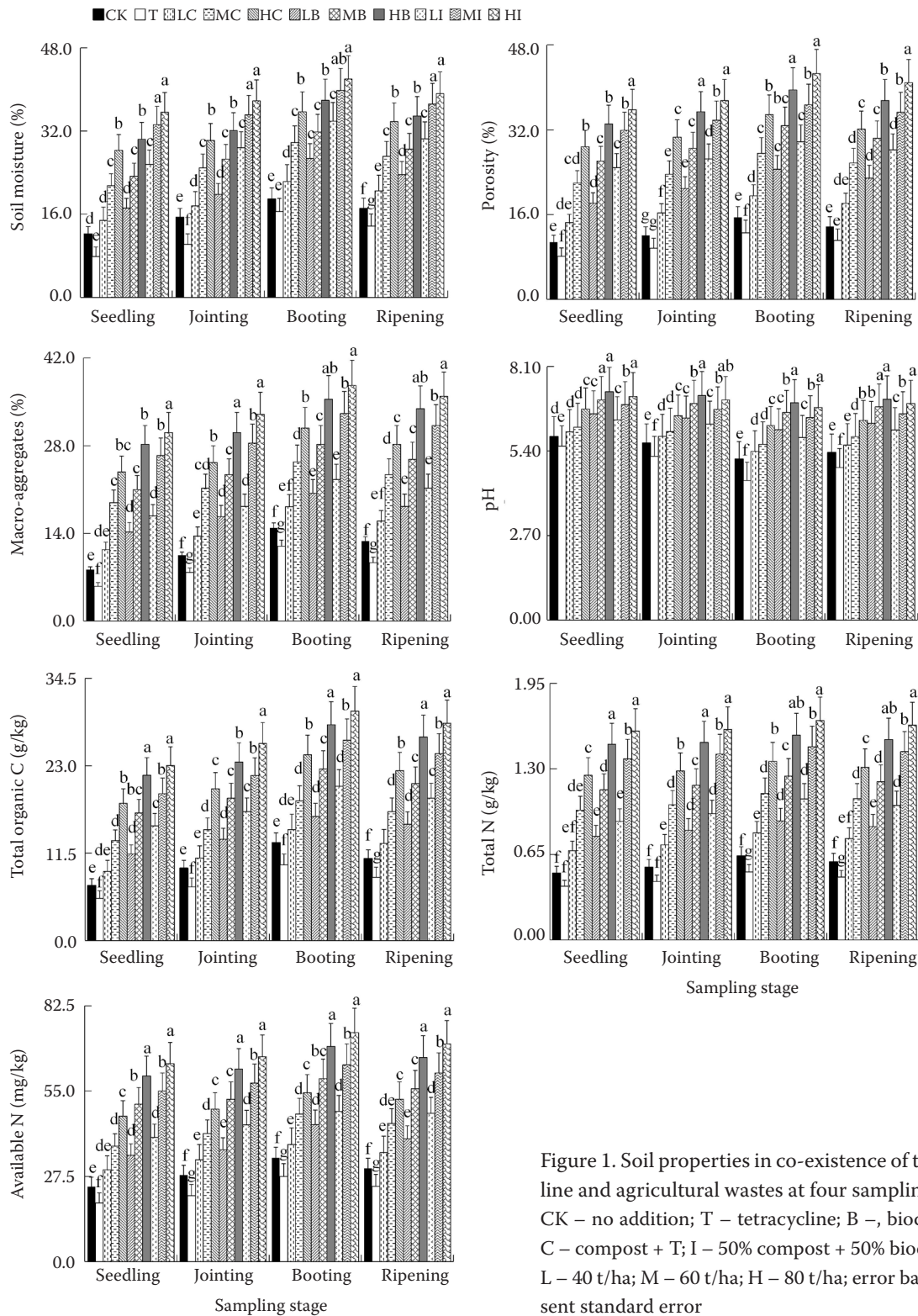


Figure 1. Soil properties in co-existence of tetracycline and agricultural wastes at four sampling stages CK – no addition; T – tetracycline; B –, biochar + T; C – compost + T; I – 50% compost + 50% biochar + T; L – 40 t/ha; M – 60 t/ha; H – 80 t/ha; error bars represent standard error

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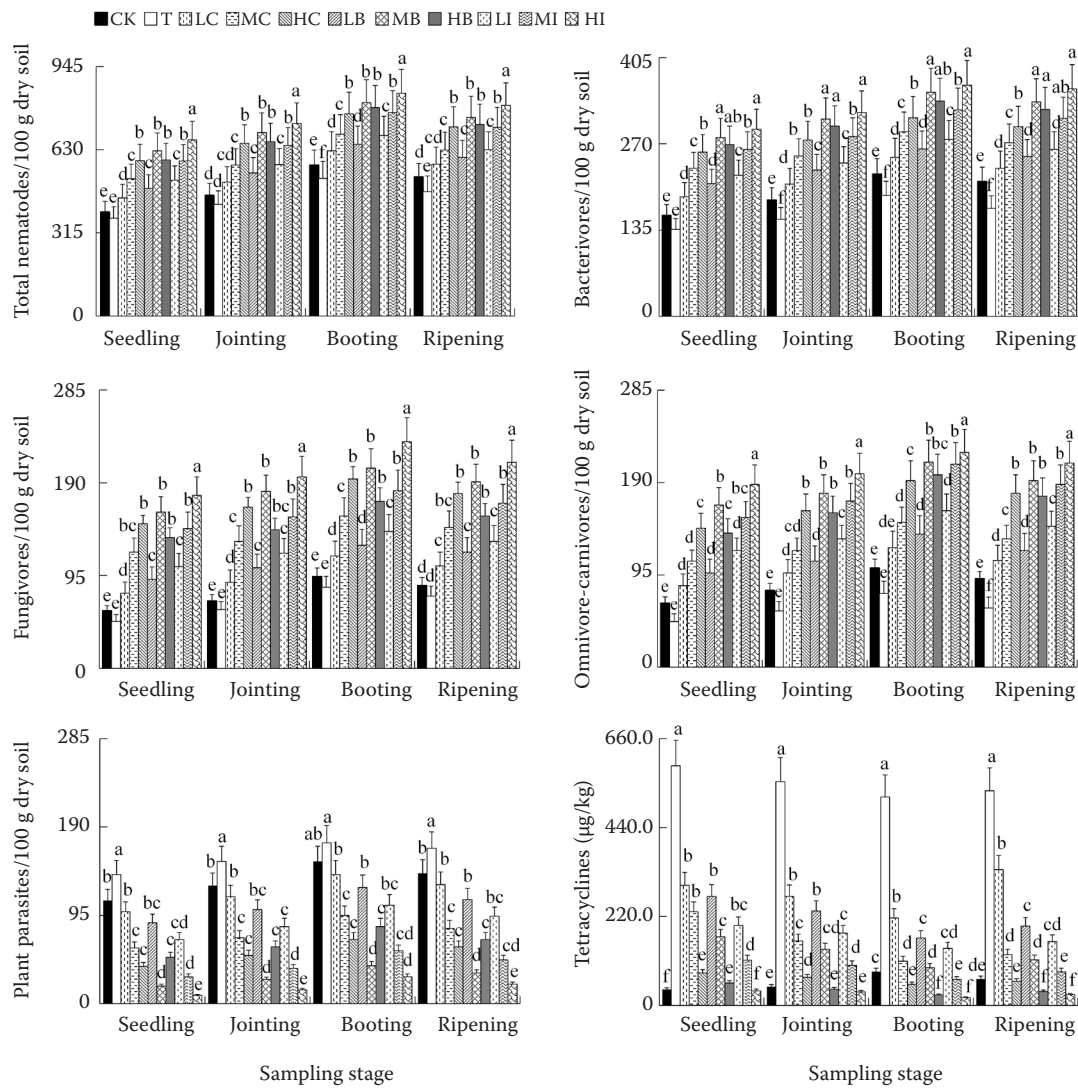


Figure 2. The abundance of nematodes in co-existence of tetracycline and agricultural wastes at four sampling stages CK – no addition; T – tetracycline; B –, biochar + T; C – compost + T; I – 50% compost + 50% biochar + T; L – 40 t/ha; M – 60 t/ha; H – 80 t/ha; error bars represent standard error

the lowest abundance in the I treatment and the highest abundance in the T treatment. The abundance of bacterivores, fungivores and omnivores significantly increased with the increase of compost additive amount (Figure 2).

**Community indicators.** Treatment and sampling effects were significant for Shannon-Weaver diversity ( $H'$ ), species richness (SR) and basal index (BI) ( $P < 0.01$ , Table 2). The highest and the lowest values of  $H'$ , SR, maturity index (MI), enrichment index (EI) and structure index (SI) were found in the I treatment and the T treatment, respectively (Figure 3).  $H'$  and SR significantly increased with the increase of the

additive amount of each amendment ( $P < 0.01$ ).  $H'$ , MI, EI and SI values approached their agroecological maximums of 3.5, 5.0, 100 and 100 and  $H'$ . MI and SI values approached their agroecological minimums of 1.5, 1.0 and 0 (Table S5 in the ESM).

Biochar input was more effective than compost to increase SI, and the mixed amendments preferred to increase EI (Figure 3). EI and SI greatly increased with the increase of biochar or compost additive amount ( $P < 0.01$ ). Channel index (CI) values greatly decreased, but BI and plant parasite index (PPI) firstly decreased and increased afterwards with the increase of biochar amendment amount. The average values of these indica-

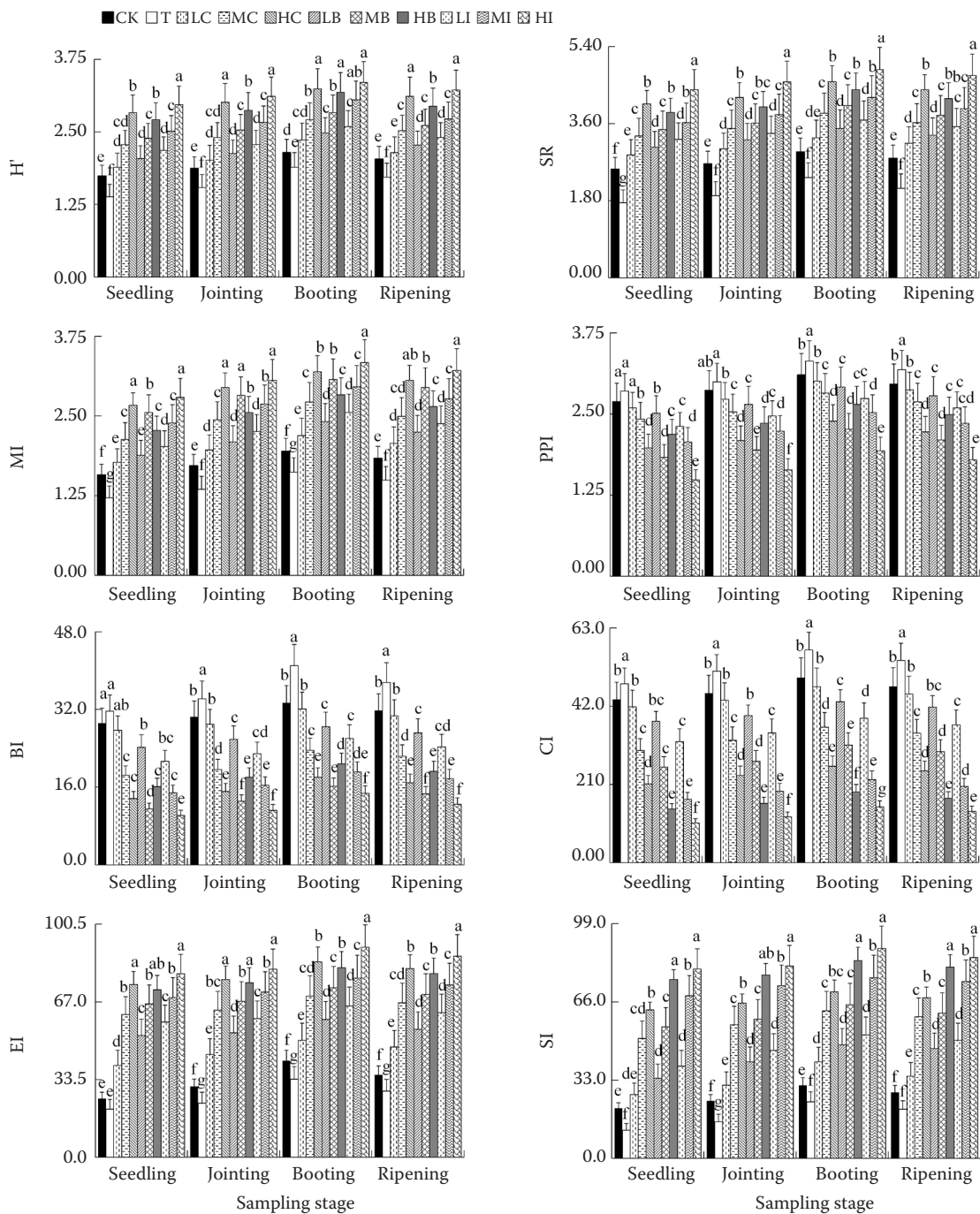


Figure 3. Nematode ecological indicators in co-existence of tetracycline and agricultural wastes at four sampling stages CK – no addition; T – tetracycline; B –, biochar + T; C – compost + T; I – 50% compost + 50% biochar + T; L – 40 t/ha; M – 60 t/ha; H – 80 t/ha; error bars represent standard error  
 H' – Shannon-Weaver diversity; SR – species richness; MI – maturity index; PPI – plant parasite index; BI – basal index; CI – channel index; EI – enrichment index; SI – structure index

tors ranged with the order: I < B < C < CK < T. All the community indicators showed the highest values in the bootling stage and the lowest of those in seedling stage, presenting a “n” fluctuation (Figure 3).

## DISCUSSION

**Effect of tetracycline on soil nematode composition.** The abundance of bacterivores were 91.2% and

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78.6% higher in the I treatments than in the T treatment and CK, respectively, which were consistent with other findings (Zheng et al. 2020; Singh et al. 2024). They found that the C/N ratio of biochar and compost combination was readily to be decomposed by tetracycline-degrading bacteria such as *Achromobacter* spp., *Petrimonas* spp. and *Bacteroides* spp. and biochar accumulated more tetracyclines for these bacteria to utilise.

The abundance of fungivores firstly increased with the increase of biochar additive amount and decreased afterwards, which was inconsistent with previous researchers (Santás-Miguel et al. 2020). They found that tetracyclines did not exert significant effects on fungivores under biochar conditions. We found that tetracycline was more likely to bind with total organic carbon under high soil pH, which decreased its bioavailability and toxicity on fungivores with c-p 4 such as *Dorylaimoides* and *Tylencholaimus*.

Carnivores-omnivores were 90.6% and 81.7% higher in I treatments than in T treatment and CK, respectively, which were consistent with previous studies (Yue et al. 2021; Zhang et al. 2022) and suggested that the mixed amendments greatly increased soil porosity and macro-aggregates, thus promoting oxygen and water penetration, and these genera were suitable for growth and reproduction in well-aerated conditions.

Plant parasites were dominant genera in the T treatment and CK. Plant pathogenic nematodes such as *Pratylenchoides*, *Criconemoides* and *Hemicycliophora* showed the lowest relative abundances in I treatments, which were consistent with other researchers (Liu et al. 2020; Zhang et al. 2022), and suggested that the combined amendments not only assisted natural enemies of plant parasites but also stimulated host roots to secrete allelochemicals such as quinones and terpenoids to repel plant parasites.

**Effect of tetracycline on ecological indicators.** H' and SR were 76.3% and 85.8% higher in the I treatments than in the T treatment, respectively, which were consistent with other organic amendments combination (Liu et al. 2023), and indicated that 60 g/ha of tetracycline had slight effects on H' and SR under biochar mixed with compost amendment. The reasons were attributed to the combinations greatly enhancing species of tetracycline degrading-bacterivores and resistant-bacterivores.

MI firstly increased with the increase of biochar additive amount and decreased afterward, while PPI showed the opposite tendency with the highest values

in 60 t/ha, which was consistent with the findings of Karamova et al. (2022), indicating that moderate biochar input partly provided a healthy and undisturbed environment for free-living nematodes and declined toxicity of tetracycline residue on these genera, partly effectively inhibited plant parasites' activity, but biochar overuse could be toxic to bacterivores and omnivores.

CI values were the highest in the T treatment and 2.23 times higher than in the I treatment, indicating that tetracycline killed more bacterivores than fungivores. Similar results were observed by Wang et al. (2024), indicating that bacterivores, especially those genera with high coloniser-persister (c-p) values, were not as tolerant as fungivores when suffering from tetracycline pollution.

BI and EI values continuously decreased and increased with the addition of amendments in I treatments, respectively, which was consistent with the results of Zeng et al. (2025) and indicated that compost input provided plentiful nutrients for bacterivores and fungivores, especially those of low c-p values and biochar input resulted in slow release of these nutrients. SI values were 79.3% and 65.9% higher in I treatments than in T treatment and CK, respectively, suggesting that free-living nematodes with c-p 3-5 were dominant genera in the combined amendments, and a less stressed and structured food web was constituted (Wang et al. 2025).

## CONCLUSION

Both biochar and compost, especially their combination, effectively improved soil physicochemical properties such as macro-aggregates, total organic C and total N. High-dose input of combinations greatly increased tetracycline-degrading nematodes, mainly including bacterivores and fungivores with low c-p values and decreased the abundance of plant pathogenic nematodes. Overall, the combined application reduced soil tetracycline residue below the threshold limit and benefited the soil nematode community structure in the soil food web under maize cultivation.

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