

Original Research

Response of Soil Greenhouse Gases Emissions to Microplastics Accompanied with Earthworms and Biochar from a Sandy-Loam Soil

Wenjing Ma¹, Jinghan Yue¹, Feng Lin², Yaojun Zhang^{1,3*}

¹International Joint Research Laboratory for Global Change Ecology, School of Life Sciences, Henan University, Kaifeng, Henan 475004, China

²School of Environmental Engineering, Nanjing Institute of Technology, Nanjing, Jiangsu 211167, China

³Yellow River Floodplain Ecosystems Research Station, School of Life Sciences, Henan University, Xingyang, China

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Abstract

Microplastics (MPs), biochar, and earthworms are critical yet understudied drivers of greenhouse gas (GHG) emissions in agricultural soils. However, limited research has explored the interactive effects of these factors on soil GHG emissions and soil carbon and nitrogen cycling. Here, we conducted a full-factorial mesocosm experiment ($2 \times 2 \times 2$ design) to assess the individual and combined influences of PVC microplastics (1% w/w), biochar (1% w/w), and the epigeic earthworm on carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄) emissions in a sandy-loam soil. The results revealed that MPs increased soil CO₂ emissions while suppressing N₂O and CH₄ emissions. Earthworms elevated CO₂ and N₂O emissions by 42.3% and 27.3%, respectively. Biochar amplified CO₂ release by 20.6% and reduced N₂O by 26.1%. The interaction between MPs and earthworms significantly influenced CO₂ emissions and the global warming potential (GWP). Both MPs and biochar significantly enhanced earthworm survival rates by 24-33% but did not affect individual biomass. Soil properties were partially influenced by the individual or combined effects of MPs, biochar, and earthworms. Overall, these results underscore the need for integrated amendment strategies to mitigate GHG emissions in MP-contaminated agroecosystems, balancing carbon sequestration priorities with soil health preservation.

Keywords: carbon dioxide, nitrous oxide, methane, MBC, MBN

Introduction

Carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄) constitute the dominant anthropogenic greenhouse gases (GHGs), collectively responsible for

over 90% of radiative forcing driving contemporary climate change [1]. Notably, the warming potentials of N₂O and CH₄ are approximately 298 and 35 times higher than that of CO₂ over a 100-year horizon, respectively [2]. Agricultural soils are critical GHG sources, emitting 5-20% of CO₂, 15-30% of CH₄, and 80-90% of N₂O annually [3]. Various factors influence soil GHG emissions, including biological (e.g., earthworms) and non-biological (e.g., microplastics, biochar) factors.

*e-mail: njayujzhang@163.com

For instance, neutral soils favor maximal CO₂ efflux, acidic soils enhance N₂O production, and alkaline soils promote CH₄ oxidation [4]. Addressing these dynamics requires a holistic assessment of biotic and abiotic drivers, including emerging contaminants and soil amendments.

Microplastics (MPs, size < 5mm), derived from plastic fragmentation, have emerged as a pervasive threat to agroecosystems [5]. MPs infiltrate soils mainly via plastic mulching and organic fertilizer, with mulching being one of the most significant sources of MPs in arable soil [6]. Their persistence and mobility enable MPs to alter soil structure, aeration, and microbial habitats, thereby disrupting carbon and nitrogen cycling [7, 8]. Meta-analyses indicate that MPs generally elevate CO₂ (+54.3%) and N₂O (+140.6%) emissions, though responses vary by polymer type, soil pH, and land use [9, 10]. For example, polyethylene MPs tripled N₂O fluxes in flooded paddy soils but suppressed CH₄ emissions in alkaline soils [11-13]. Such context-dependent effects underscore the need to evaluate MPs' interactions with co-occurring soil amendments.

Biochar, a carbon-rich pyrolysis product, is widely advocated for soil carbon sequestration and GHG mitigation [14]. Its porous structure and alkaline nature enhance nutrient retention, microbial activity, and soil aggregation [15]. However, biochar's impact on GHG emissions remains contentious: global syntheses report CH₄ and N₂O reductions of 9-72% and 14-60%, respectively [16-18], while other studies document increased CO₂ emissions or null effects [19-21]. These discrepancies likely arise from feedstock-specific properties (e.g., lignin content, pyrolysis temperature) and soil type [22].

As ecosystem engineers, earthworms further modulate GHG dynamics through bioturbation, organic matter mineralization, and gut-associated microbial processes [23]. Previous studies have demonstrated that the presence of earthworms can exert both positive

and negative effects on soil GHG emissions [24]. For example, in a two-year microcosmic experiment, they reported that earthworms did not contribute to GHG emissions [25]. Conversely, the presence of earthworms notably increased N₂O, and CO₂ emissions were also certified [24]. These contrasts highlight knowledge gaps regarding earthworm interactions with MPs and biochar—a critical oversight given their co-occurrence in agricultural systems.

Despite advances in understanding individual drivers, the synergistic or antagonistic effects of MPs, biochar, and earthworms on GHG emissions remain unexplored. Preliminary evidence suggests biochar may offset MP-induced N₂O emissions in earthworm-inhabited acidic soils [26], but no studies have examined these interactions in alkaline vertisols. Here, we address this gap through a controlled mesocosm experiment using sandy-loam soil. We hypothesized that (1) MPs and earthworms would synergistically enhance CO₂ and N₂O emissions by accelerating organic matter turnover and nitrogen mineralization, and (2) biochar would mitigate MP/earthworm-driven GHG fluxes via carbon stabilization and microbial community modulation. To validate these hypotheses, we measured the emissions of CO₂, N₂O, and CH₄ from sandy-loam soil under the addition of MPs, earthworms, and biochar.

Materials and Methods

Soil Sampling, Biochar, MPs, and Earthworm Preparation

Soil samples (0–20 cm depth) were collected from a sandy-loam vertisol (USDA soil classification) under a decade-long maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) rotation at the Sustainable Agro-Ecology Experimental Station of Henan University, Kaifeng, China (34° 39' N, 114° 14' E, 76 m a.s.l.). The soil,

Table 1. Soil, microplastic, and biochar physicochemical properties (mean ± standard deviation) before the incubation.

Properties	Soil	Microplastics	Biochar
	Value		
Clay (%)	11.7±0.6	-	-
Silt (%)	19.7±1.1	-	-
Sand (%)	68.6±1.2	-	-
Total C (mg kg ⁻¹)	236.6±2.5	86.2±1.1	435.6±1.2
Total N (mg kg ⁻¹)	26.5±0.5	0.03±0.0	6.4±0.6
C-to-N ratio	8.9	2873	68.1
NH ₄ ⁺ -N (mg kg ⁻¹)	6.5±0.9	-	-
NO ₃ ⁻ -N (mg kg ⁻¹)	26.2±3.4	-	-
pH	8.5±0.03	7.3±0.05	10.7±0.02
Bulk density (g cm ⁻³)	1.26	0.88	0.52

Table 2. Earthworm survival rate after 21 days.

Treatment	Individual (individual pot ⁻¹)		Biomass (g individual ⁻¹)	
	Beginning	Ending	Beginning	Ending
E-M0	-	-	-	-
E+M0	15	10.50±0.29 b	0.43	0.28±0.01 a
E-M10	-	-	-	-
E+M10	15	14.00±0.41 a	0.44	0.26±0.01 a
B+E-M0	-	-	-	-
B+E+M0	15	13.00±1.08 a	0.44	0.26±0.01 a
B+E-M10	-	-	-	-
B+E+M10	15	14.00±0.91 a	0.44	0.27±0.01 a

derived from Yellow River alluvium, comprised 68.6% sand, 19.7% silt, and 11.7% clay. Visible plant debris and any fragments were removed. Samples were air-dried at $25 \pm 2^\circ\text{C}$ for 14 days, homogenized, and sieved through a 2-mm stainless steel mesh prior to use. The biochar used in this study was derived from wheat straw via pyrolysis at 550°C . Polyvinyl chloride (PVC) microplastics (MPs; <0.5 mm diameter) were sourced from Wangda Plastic Co. (Dongguan, China). The primary properties of the soil, biochar, and MPs are presented in Table 1.

The epigeic earthworm *Eisenia fetida* was selected for this investigation due to its prevalence in regional agroecosystems and standardized use in ecotoxicological assays [26-27]. The earthworms were carefully washed, dried on filter paper, and weighed prior to their introduction into each experimental pot. Detailed information on the earthworms is provided in Table 2.

Experimental Design and Setup

A full-factorial mesocosm experiment ($2 \times 2 \times 2$ design) was conducted to assess the individual and interactive effects of PVC-MPs (MPs: 0% [M0] or 1% w/w [M10]), biochar (0% [B-] or 1% w/w [B+]), and earthworms (0 [E-] or 15 individuals pot⁻¹ [E+]). Each treatment combination was replicated four times ($n = 32$ pots total). Mesocosms consisted of 6 L polypropylene pots filled with 7.0 kg of air-dried sandy-loam vertisol (sieved to 2 mm), homogenized with MPs and/or biochar. Earthworms (4.8–5.0 g individual⁻¹) were surface-sterilized with clean water, rinsed, and acclimated for 48 h prior to introduction. Pots were maintained at $20 \pm 2^\circ\text{C}$ under 60% water-holding capacity for 21 days, with fleece-lined bases to prevent earthworm escape.

Greenhouse Gas Measurement

GHG fluxes were measured at 1, 2, 3, 4, 6, 7, 10, 13, and 21 days using static chambers (50 cm height \times 40 cm diameter) sealed to pots via water-filled grooves. Gas

samples (20 mL) were collected at 0 and 120 min using gas-tight syringes, stored in pre-evacuated vials, and analyzed within 24 h via gas chromatography (Agilent 7890B). CO_2 and CH_4 were quantified using flame ionization detection (FID; 250°C detector temperature) and N_2O using electron capture detection (ECD; 350°C). The GWP for a 100-year time horizon, including climate-carbon feedbacks, was calculated using a radiative forcing potential relative to CO_2 of 34 for CH_4 and 298 for N_2O .

Soil Analysis

Following a 21-day incubation period, soil samples were analyzed. Soil pH was measured in a 1:2.5 (w/v) soil-deionized water suspension using a calibrated pH meter (Orion Star A329, Thermo Scientific). Dissolved organic carbon (DOC) and total nitrogen (TN) soil extracts (2 M KCl, 1:5 w/v) were shaken (200 rpm, 25°C , 1 h), centrifuged ($4,000 \times g$, 15 min), and filtered (0.45 μm GF/C membranes). DOC and TN concentrations were quantified via high-temperature combustion (Shimadzu TOC-VCSH/TNM-1; detection limits: 0.1 mg C L⁻¹, 0.01 mg N L⁻¹). Mineral nitrogen: NH_4^+ -N and NO_3^- -N in filtered extracts were analyzed colorimetrically using a Flow Injection Analyzer (Lachat QuikChem 8500; detection limits: 0.01 mg N L⁻¹). The chloroform fumigation-extraction method determined the soil microbial biomass carbon (MBC) and nitrogen (MBN). Briefly, fumigated (24 h, CHCl_3 vapor) and non-fumigated soils were extracted with 0.5 M K_2SO_4 (1:4 w/v). MBC and MBN were calculated as [(fumigated – non-fumigated C or N) / 0.45 and 0.54, respectively].

Data Analysis and Statistics

Three-way ANOVA with Tukey's HSD post-hoc tests ($\alpha = 0.05$) assessed main and interactive effects using the SPSS 27 package. Data met normality (Shapiro-Wilk) and homogeneity (Levene's test) assumptions; non-normal datasets were log-transformed. Treatment

Table 3. Soil characteristics after the 21-day incubation (mean values \pm standard error). Different letters within each treatment indicate significant differences for the Fisher LSD test.

Treatments	Variables									
	pH	NH ₄ ⁺ -N (mg kg ⁻¹)	NO ₃ ⁻ -N (mg kg ⁻¹)	DOC (mg kg ⁻¹)	TN (mg kg ⁻¹)	DOC:TN	MBC (mg kg ⁻¹)	MBN (mg kg ⁻¹)	MBC:MBN	
Microplastic (MP)										
Control	8.45 \pm 0.03a	6.07 \pm 0.44a	33.12 \pm 2.31a	271.10 \pm 10.61b	27.11 \pm 0.27b	10.00 \pm 0.38b	84.64 \pm 9.11b	19.03 \pm 1.86b	4.55 \pm 0.37b	
+MP	8.48 \pm 0.04a	6.34 \pm 0.34a	23.53 \pm 2.43b	434.55 \pm 22.08a	29.64 \pm 0.38a	14.58 \pm 0.59a	137.83 \pm 7.33a	23.01 \pm 1.58a	6.29 \pm 0.41a	
Earthworm (E)										
Control	8.54 \pm 0.03a	6.30 \pm 0.35a	21.90 \pm 1.86b	348.44 \pm 23.16a	27.96 \pm 0.43a	12.35 \pm 0.66a	122.46 \pm 10.77a	21.90 \pm 0.98a	5.63 \pm 0.42a	
+E	8.38 \pm 0.02b	6.10 \pm 0.43a	34.75 \pm 2.32a	357.21 \pm 30.85a	28.79 \pm 0.47a	12.23 \pm 0.87a	100.01 \pm 9.91b	20.14 \pm 2.33a	5.21 \pm 0.47a	
Biochar (B)										
Control	8.39 \pm 0.02b	6.06 \pm 0.44a	27.22 \pm 2.68a	305.19 \pm 19.27b	27.94 \pm 0.38a	10.84 \pm 0.57b	90.14 \pm 10.59b	20.33 \pm 2.15a	4.47 \pm 0.33b	
+B	8.54 \pm 0.03a	6.35 \pm 0.34a	29.43 \pm 2.65a	400.45 \pm 28.58a	28.82 \pm 0.51a	13.74 \pm 0.77a	132.32 \pm 7.72a	21.70 \pm 1.34a	6.36 \pm 0.42a	
ANOVA										
Microplastic	0.0501	0.607	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Earthworm	<0.001	0.693	<0.001	0.611	0.063	0.804	<0.001	0.188	0.296	
Biochar	<0.001	0.577	0.374	<0.001	0.051	<0.001	<0.001	0.304	<0.001	
MP \times E	0.545	<0.05	0.857	0.099	0.625	0.084	0.264	0.397	0.934	
MP \times B	0.078	0.081	0.289	0.183	0.200	0.435	<0.001	<0.001	0.579	
E \times B	0.657	0.408	0.472	0.788	0.895	0.934	0.214	0.776	0.855	
MP \times E \times B	<0.001	0.979	0.149	0.207	0.372	0.219	<0.01	<0.001	<0.01	

effects were quantified as percentage changes relative to controls (95% confidence intervals).

Results and Discussion

Earthworm Survival and Soil Biogeochemical Properties

The survival of earthworms across all treatments was not optimistic, although they remained alive throughout the incubation period (Table 2). E+M0 treatment especially exhibited the lowest earthworm survival rate of 53% compared to the initial state. Notably, the incorporation of microplastics or biochar increased survival by 24-33% ($p < 0.05$), likely due to MPs improving soil aggregation (enhancing habitat structure) and biochar buffering moisture fluctuations. Despite these improvements, individual earthworm biomass declined uniformly by 35-41% ($p > 0.05$), suggesting physiological stress from MP exposure, potentially via oxidative damage or gut obstruction.

Soil pH exhibited dual regulation: earthworms reduced pH by 1.9% (8.54 vs 8.38; $p < 0.01$) through acidifying excretions (e.g., ammonium), while biochar increased it by 1.8% (8.39 vs 8.54; $p < 0.001$) via its alkaline ash content (Table 3). This pH modulation influenced microbial community composition, favoring nitrifying bacteria under biochar-amended conditions. MPs reduced NO_3^- -N by 29.0% ($p < 0.01$), likely by adsorbing NO_3^- on hydrophobic surfaces, whereas earthworms increased NO_3^- -N by 58.7% ($p < 0.001$) via enhanced mineralization of organic N (Table 3). The MP \times E interaction amplified NH_4^+ -N depletion ($p < 0.05$), reflecting earthworm-driven nitrification of NH_4^+ -N in MP-aerated soils. MPs elevated DOC by 60.3% ($p < 0.001$) and DOC:TN by 45.8% ($p < 0.01$) (Table 3), directly correlating with CO_2 emissions. Biochar increased DOC by 22.1% ($p < 0.05$), likely through leaching of labile C fractions. Microbial biomass responded divergently: MPs and biochar synergistically increased MBC by 28.4% ($p < 0.01$) but reduced MBN by 19.7% ($p < 0.05$), elevating MBC:MBN by 32.6% ($p < 0.01$). This shift suggests N-limitation for microbial growth, suppressing nitrifier activity and N_2O production.

Greenhouse Gas Flux Dynamics

The three greenhouse gases exhibited distinct temporal flux dynamics (Fig. 1). CO_2 fluxes declined sharply within 132 hours, followed by slight fluctuations at low rates until the end of the incubation. Adding earthworms and biochar significantly enhanced CO_2 emissions. Across all treatments, N_2O flux emissions peaked in the first 4 days (~84 h) of incubation and then declined steadily thereafter. Some negative N_2O fluxes were observed between 300 and 516 hours. In contrast, CH_4 emissions were relatively variable and did

not exhibit a clear pattern. Furthermore, CH_4 emissions were negative for all the treatments, indicating net CH_4 oxidation.

A substantial amount of CO_2 emissions occurred in the initial stage and then decreased steadily. This is likely due to the addition of MPs and biochar providing an external input of available C for heterotrophic consumption of labile C, while exhaustion of labile C thereafter leads to a steady decrease [28]. Furthermore, earthworms generally lead to higher CO_2 emissions, as their activity in soil facilitates the availability of more oxygen for CO_2 production and release [29]. Additionally, biochar amendment resulted in higher CO_2 emissions than the control in the initial stages (Fig. 1c), primarily due to the increased soil organic C mineralization induced by its priming effect [30]. N_2O emission fluxes generally followed a similar trend but exhibited varied responses to the three factors during the first 10 days of the incubation. These results are likely attributed to two distinct processes: 1) adding MPs or biochar enhancing soil C/N ratio and promoting ammonia oxidation [31], and 2) earthworm activity accelerating the availability of soil mineral N, which is associated with soil nitrification and denitrification processes [32]. CH_4 fluxes were almost negative for all the treatments, which means the sandy-loam alkaline soil here has an intense capacity for CH_4 oxidation [33].

Effect of MPs on GHG Emissions

Adding MPs exerted significant yet inconsistent effects on the three greenhouse gas emissions (Fig. 2, Table 4). Specifically, it significantly increased CO_2 emissions and GWP while decreasing N_2O and CH_4 emissions. The different effects of MPs on these three GHG emissions may stem from various potential reasons. Firstly, adding MPs improves soil porosity, aggregation, and aeration [34, 35], facilitating the diffusion of O_2 and CO_2 from the soil [36]. It is known that N_2O production primarily depends on denitrification under anaerobic conditions and nitrification under aerobic conditions [37]. Soil O_2 increase can reduce microbial denitrification, decreasing soil N_2O emissions. Additionally, the increased soil O_2 content also accelerates CH_4 oxidation and restrains soil CH_4 emission [38]. Secondly, the soil C:N ratio is a crucial indicator for assessing soil GHG emissions, particularly in relation to N_2O emissions [39]. Previous studies have demonstrated a robust inverse correlation between the soil C/N ratio and N_2O emission [40], consistent with our findings. Furthermore, 22%-46% of the DOC in MPs can be utilized by organisms or microorganisms in the carbon cycle as energy or nutrients [41], correlating with the increase in CO_2 emission and the decrease in N_2O emission. Thirdly, a higher MBC:MBN ratio primarily impeded soil nitrification, subsequently reducing N_2O emission [42]. A recent global analysis revealed a negative correlation between the MBC:MBN ratio and soil N_2O emissions [43]. Furthermore, previous

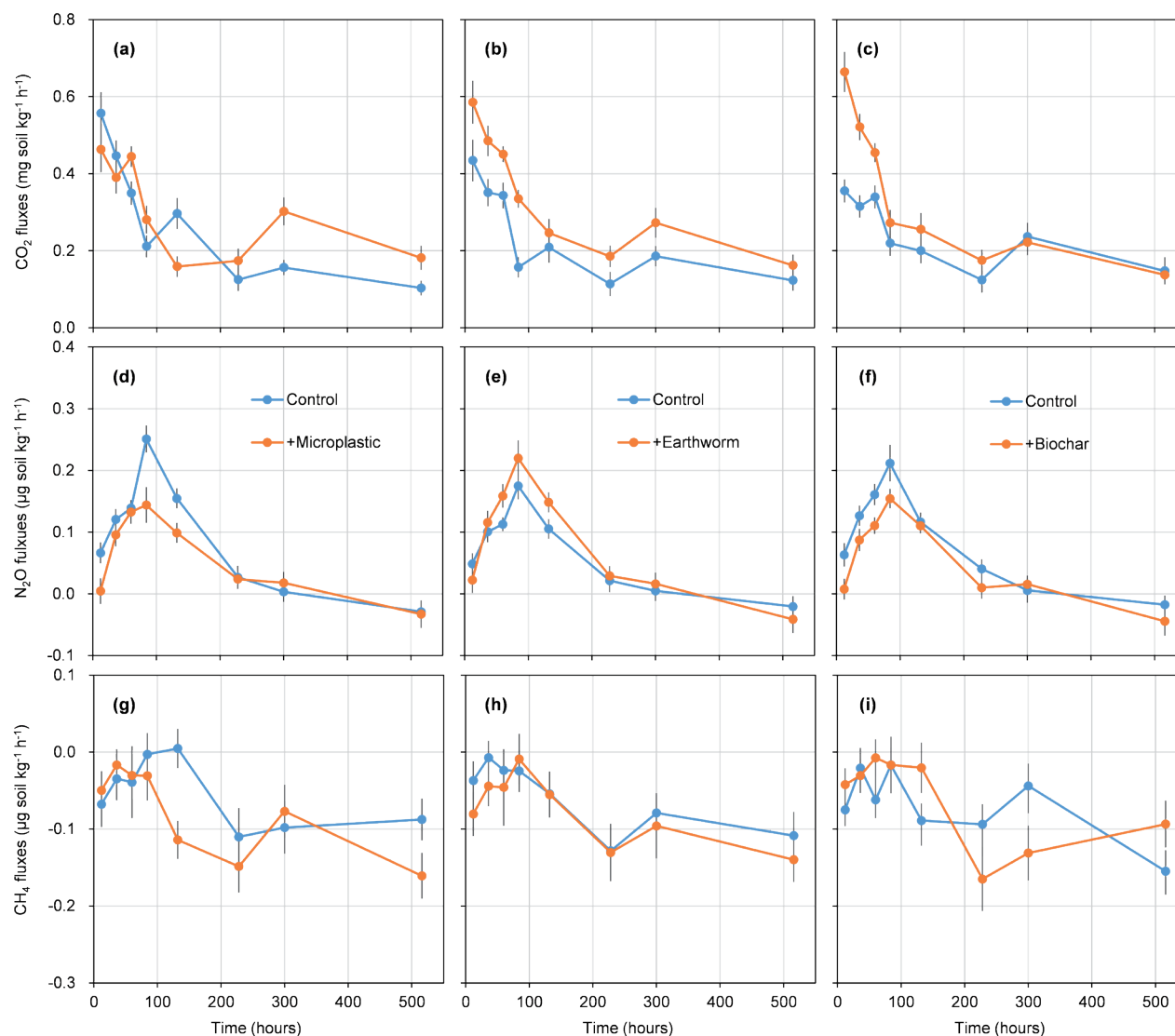


Fig. 1. Hourly CO_2 (a, b, and c), N_2O (d, e, and f), and CH_4 (g, h, and i) emission fluxes during the 21-day incubation. The error bars indicate the standard errors of means ($\pm\text{SE}$, $n=16$).

research discovered a strong correlation between soil N_2O emissions and NO_3^- content in spatial and temporal models [44]. This suggests that a reduction in NO_3^- levels would decrease inorganic nitrogen availability for microbial denitrification, ultimately leading to a decrease in N_2O emissions as an intermediate product.

Effect of Earthworms on GHG Emissions

The presence of earthworms in the soil significantly increased soil CO_2 and N_2O emissions. The presence of earthworms significantly enhanced GWP by 50.9% compared to the control. However, earthworms did not affect CH_4 production (Fig. 2, Table 4). This effect was likely due to the changes in soil properties caused by earthworms and their inherent characteristics. Earthworm respiration contributes significantly to soil respiration [26], accounting for the 42.3% increase in CO_2 emissions observed in our study due to the presence

of earthworms. This result is similar to the findings of a 34% increase in CO_2 emissions due to the presence of earthworms [45, 46]. Our results indicated that the presence of earthworms had a strong influence on accelerating N_2O emissions, which is consistent with previous studies [47]. This phenomenon is likely due to the increase in soil NO_3^- content, which provides sufficient substrate for denitrification and thus stimulates soil N_2O emissions [48]. In the current study, the presence of earthworms did not affect CH_4 emissions, a finding that is supported by previous studies [49]. However, there are differing results regarding the effect of earthworms on CH_4 emissions. For instance, aquatic earthworms could reduce CH_4 fluxes by promoting a favorable environment for methanotrophs [50].

Effect of Biochar on GHG Emissions

Biochar amendment had a significant but opposite effect on CO_2 and N_2O emissions while having no effect

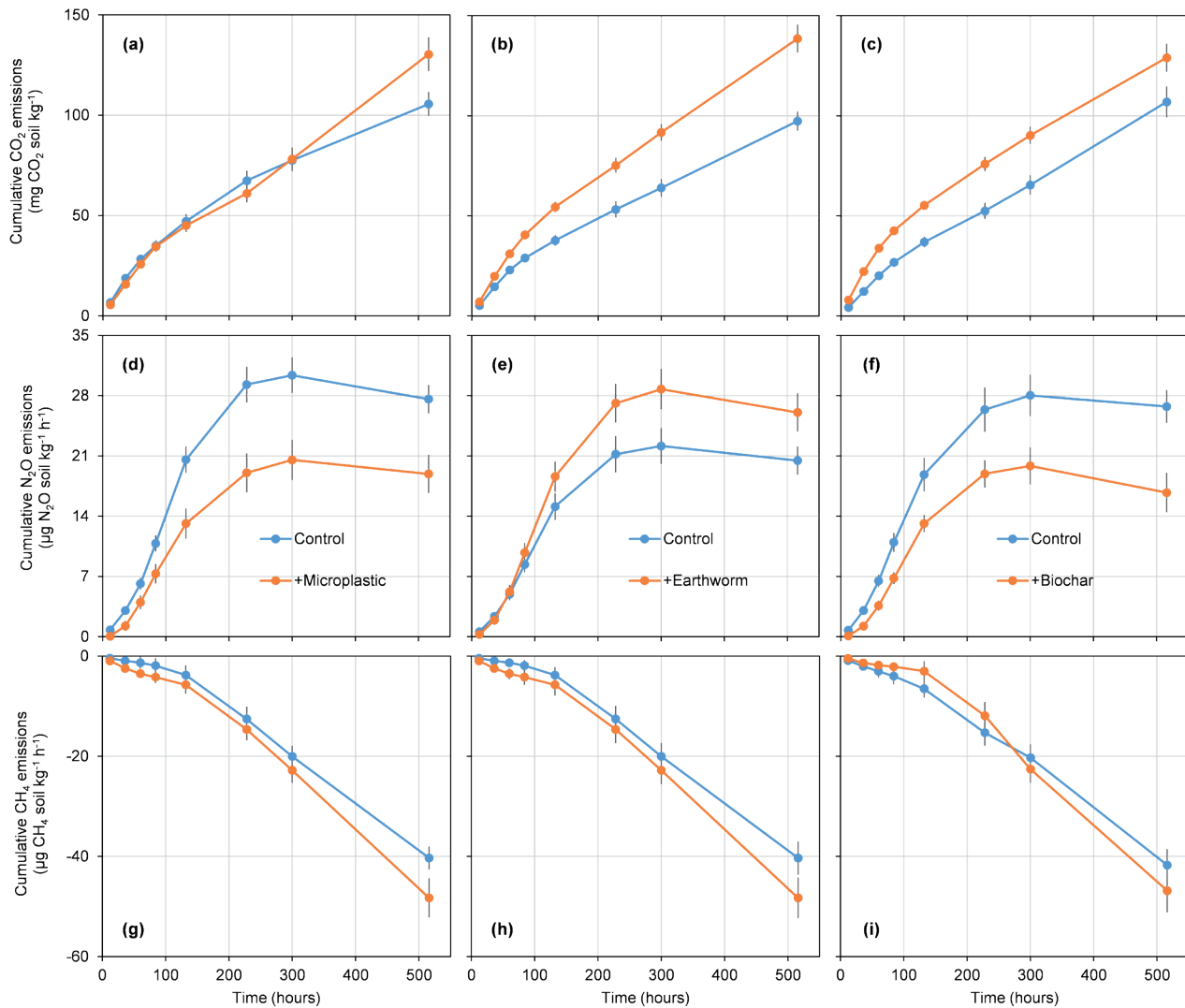


Fig. 2. Cumulative CO_2 (a, b, and c), N_2O (d, e, and f), and CH_4 (g, h, and i) emissions during the 21-day incubation. The error bars indicate the standard errors of means ($\pm\text{SE}$, $n=16$).

on CH_4 production (Fig. 2, Table 4). Specifically, biochar amendment significantly increased CO_2 emissions while decreasing N_2O emissions relative to the unamended treatments.

Biochar is a highly effective material for regulating soil's physico-chemical environment, given that GHG emissions are closely correlated with soil physical properties (e.g., pH, porosity, aggregation) and chemical properties (e.g., carbon-nitrogen ratio, microbial carbon) [51]. Our study found that biochar amendment significantly impacted several soil properties, including pH, DOC, and MBC, thereby influencing soil CO_2 and N_2O emissions. Previous studies reported that biochar amendment could increase CO_2 emissions by 22.14% and decrease N_2O emissions by 38% [52, 53]. These findings align closely with our own results, demonstrating a 20.6% increase in CO_2 emissions and a 26.1% decrease in N_2O emissions. Biochar has a carbon content exceeding 90%, and its addition results in higher

mineralization of labile C and inorganic C release, which stimulates soil microbial activities, ultimately promoting CO_2 emissions [54]. Furthermore, adding biochar to soil enhances soil aeration, which not only facilitates CO_2 diffusion but also suppresses microbial denitrification, thereby leading to a reduction in N_2O emissions [55]. Additionally, the higher carbon-to-nitrogen ratio in soil resulting from biochar amendment could also restrain N_2O emissions. The increased nitrogen demand by microorganisms elevates the soil carbon-to-nitrogen ratio, a crucial factor affecting nitrification and denitrification processes, ultimately decreasing N_2O emissions [56].

Comprehensive Effects of the Three Factors on GHG Emissions

Our findings revealed few interactive effects of the treatments on soil GHG emissions. Only CO_2 emissions and GWP were significantly influenced by

Table 4. Cumulative greenhouse gas emission during the 21-day incubation (mean values \pm standard error). Different letters within each treatment indicate significant differences for the Tukey HSD test.

Treatments	Variables						
	CO ₂ (mg CO ₂ kg ⁻¹)	N ₂ O (μg N ₂ O kg ⁻¹)	CH ₄ (μg CH ₄ kg ⁻¹)	GWP (mg CO ₂ (eq) kg ⁻¹)			
Microplastic (MP)							
Control	105.57 \pm 5.98	27.60 \pm 1.94	-36.02 \pm 2.28	112.57 \pm 5.95	a		b
+MP	130.36 \pm 8.44	18.91 \pm 1.57	-52.60 \pm 3.95	134.20 \pm 8.51	b		a
Earthworm (E)							
Control	97.35 \pm 4.80	20.46 \pm 1.65	-40.33 \pm 3.34	102.08 \pm 4.51	b		b
+E	138.57 \pm 6.91	26.05 \pm 2.23	-48.29 \pm 4.09	154.02 \pm 5.03	a		a
Biochar (B)							
Control	106.93 \pm 7.81	26.74 \pm 1.90	-41.76 \pm 3.22	113.48 \pm 7.76	a		b
+B	129.00 \pm 7.08	19.77 \pm 1.87	-46.86 \pm 4.32	133.29 \pm 7.07	b		a
ANOVA							
	<i>p</i> values						
Microplastic	<0.001	<0.001	<0.01	<0.01			
Earthworm	<0.001	<0.05	0.101	<0.001			
Biochar	<0.01	<0.01	0.285	<0.01			
MP \times E	<0.05 [†]	0.467	0.634	<0.05 [†]			
MP \times B	0.68	0.785	0.923	0.707			
E \times B	0.76	0.834	0.657	0.755			
MP \times E \times B	0.95	0.747	0.905	0.975			

Note: † are significant interactions, where significant differences are shown in Fig. 3.

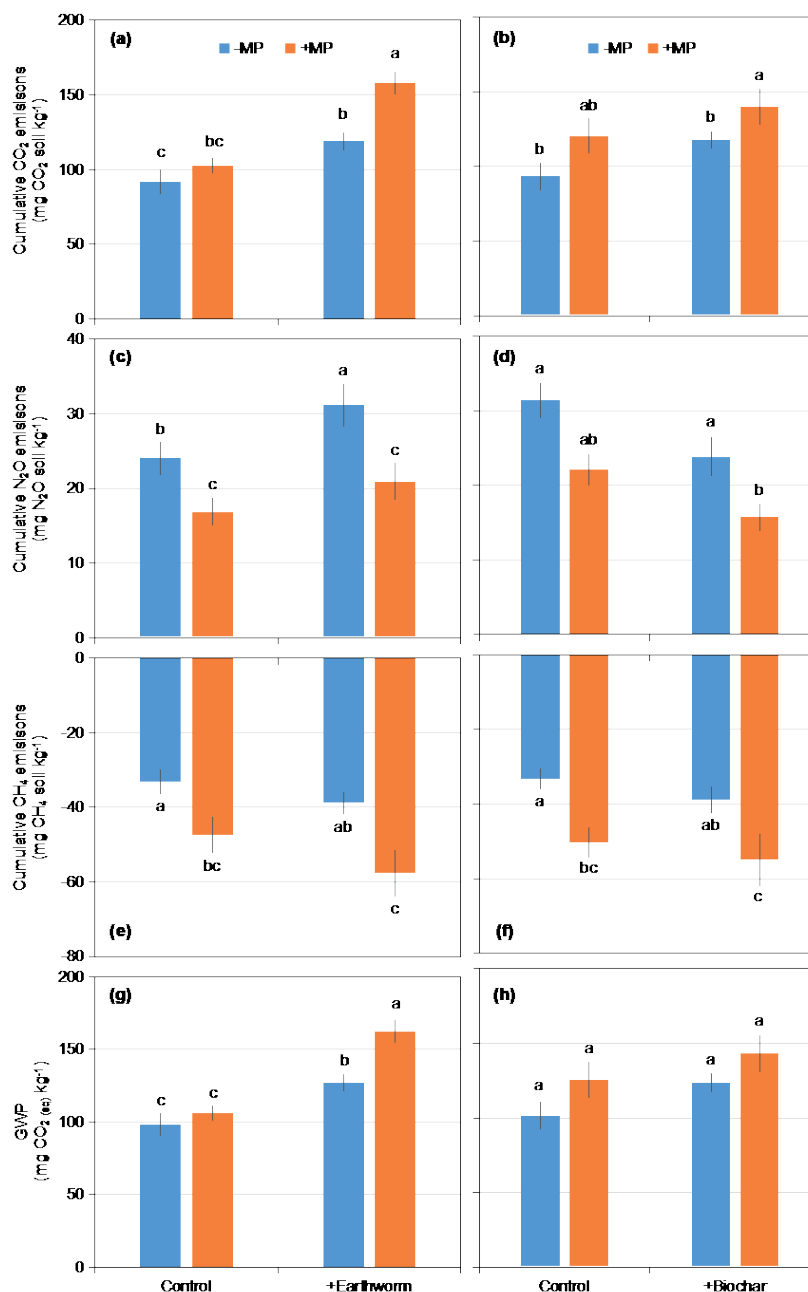


Fig. 2. Cumulative CO₂ (a, b, and c), N₂O (d, e, and f), and CH₄ (g, h, and i) emissions during the 21-day incubation. The error bars indicate the standard errors of means (\pm SE, n=16).

the interaction of MPs and earthworms (Table 4, Fig. 3). Specifically, when earthworms were absent, there was no significant difference in CO₂ emissions with or without MPs. However, the presence of earthworms significantly enhanced CO₂ emissions by 32.7% when MPs were added compared to the treatment without adding MPs (Fig. 3a). This was primarily attributed to the stimulation of earthworms' respiration by adding MPs, which is linked to soil respiration, resulting in a significant increase in soil CO₂ emissions [26]. Previous research has demonstrated that adding MPs can promote soil aeration, which favors earthworms' activities such as burrowing, feeding, and excretion [57]. Furthermore, MPs increased the survival rate of earthworms in our

study, factors that collectively enhance the respiration of earthworms themselves, ultimately increasing soil CO₂ emission. Similarly, earthworms significantly enhanced GWP by 27.7% when adding MPs (Fig. 3g). This interaction was closely associated with the combined contribution of CO₂, N₂O, and CH₄ emissions under different treatments [58]. However, the absence of earthworms resulted in no significant difference in GWP regardless of adding MPs. Currently, research on the interactive effects of earthworms and MPs on the GWP of agricultural soils is relatively limited, and the underlying mechanisms are complex, warranting further investigation in future studies.

Conclusions

This study elucidates the distinct and interactive effects of biochar, earthworms, and MPs on soil GHG emissions in a sandy-loam vertisol. Key findings reveal those alterations in soil DOC, nitrogen dynamics (TN, NO₃⁻-N), microbial biomass (MBC:MBN ratio), and C/N stoichiometry collectively drive GHG flux patterns. Our findings revealed that the survival rate, rather than the biomass, of earthworms was enhanced in the presence of earthworms and biochar. Notably, earthworms, MPs, and biochar alone significantly increased soil CO₂ emissions and GWP. Furthermore, the changes in GHG emissions under different treatments exhibited variability. Specifically, MPs decreased N₂O emissions, while earthworms and biochar increased them. Conversely, CH₄ emissions were reduced only in the presence of MPs, with no significant effect observed under earthworms or biochar addition. The interaction between earthworms and MPs (E × MP) was the only significant interaction among all treatments, which substantially influenced CO₂ emissions and GWP. These findings provide valuable insights for formulating emission reduction management strategies in sandy loam soils, aiming to mitigate the global warming potential and promote sustainable soil environmental management.

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Conflict of Interest

The authors declare no conflict of interest.

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