Pol. J. Environ. Stud. Vol. XX, No. X (XXXX), 1-12

DOI: *10.15244/pjoes/203764* ONLINE PUBLICATION DATE: 2025-12-03

Original Research

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

Wenjing Ma[1], Jinghan Yue[1], Feng Lin[2], Yaojun Zhang[1, 3]*

[1]International Joint Research Laboratory for Global Change Ecology, School of Life Sciences, Henan University, Kaifeng, Henan 475004, China
[2]School of Environmental Engineering, Nanjing Institute of Technology, Nanjing, Jiangsu 211167, China
[3]Yellow River Floodplain Ecosystems Research Station, School of Life Sciences, Henan University, Xingyang, China

Received: 24 December 2024
Accepted: 6 April 2025

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 (2×2×2 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 (CO_2), nitrous oxide (N_2O), and methane (CH_4) emissions in a sandy-loam soil. The results revealed that MPs increased soil CO_2 emissions while suppressing N_2O and CH_4 emissions. Earthworms elevated CO_2 and N_2O emissions by 42.3% and 27.3%, respectively. Biochar amplified CO_2 release by 20.6% and reduced N_2O by 26.1%. The interaction between MPs and earthworms significantly influenced CO_2 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 (CO_2), nitrous oxide (N_2O), and methane (CH_4) 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 N_2O and CH_4 are approximately 298 and 35 times higher than that of CO_2 over a 100-year horizon, respectively [2]. Agricultural soils are critical GHG sources, emitting 5-20% of CO_2, 15-30% of CH_4, and 80-90% of N_2O annually [3]. Various factors influence soil GHG emissions, including biological (e.g., earthworms) and non-biological (e.g., microplastics, biochar) factors.

---

*e-mail: njauyjzhang@163.com

For instance, neutral soils favor maximal CO_2 efflux, acidic soils enhance N_2O production, and alkaline soils promote CH_4 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 CO_2 (+54.3%) and N_2O (+140.6%) emissions, though responses vary by polymer type, soil pH, and land use [9, 10]. For example, polyethylene MPs tripled N_2O fluxes in flooded paddy soils but suppressed CH_4 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 CH_4 and N_2O reductions of 9-72% and 14-60%, respectively [16-18], while other studies document increased CO_2 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 N_2O, and CO_2 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 N_2O 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 CO_2 and N_2O 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 CO_2, N_2O, and CH_4 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 ± 2°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 × 2 × 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 ± 2°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 × 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 × 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 (α = 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 ± 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±0.03a	6.07±0.44a	33.12±2.31a	271.10±10.61b	27.11±0.27b	10.00±0.38b	84.64±9.11b	19.03±1.86b	4.55±0.37b
+MP	8.48±0.04a	6.34±0.34a	23.53±2.43b	434.55±22.08a	29.64±0.38a	14.58±0.59a	137.83±7.33a	23.01±1.58a	6.29±0.41a
Earthworm (E)									
Control	8.54±0.03a	6.30±0.35a	21.90±1.86b	348.44±23.16a	27.96±0.43a	12.35±0.66a	122.46±10.77a	21.90±0.98a	5.63±0.42a
+E	8.38±0.02b	6.10±0.43a	34.75±2.32a	357.21±30.85a	28.79±0.47a	12.23±0.87a	100.01±9.91b	20.14±2.33a	5.21±0.47a
Biochar (B)									
Control	8.39±0.02b	6.06±0.44a	27.22±2.68a	305.19±19.27b	27.94±0.38a	10.84±0.57b	90.14±10.59b	20.33±2.15a	4.47±0.33b
+B	8.54±0.03a	6.35±0.34a	29.43±2.65a	400.45±28.58a	28.82±0.51a	13.74±0.77a	132.32±7.72a	21.70±1.34a	6.36±0.42a
ANOVA									
Microplastic	0.0501	0.607	<0.01	<0.001	<0.001	<0.001	<0.001	<0.01	<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 × E	0.545	<0.05	0.857	0.099	0.625	0.084	0.264	0.397	0.934
MP × B	0.078	0.081	0.289	0.183	0.200	0.435	<0.001	<0.001	0.579
E × B	0.657	0.408	0.472	0.788	0.895	0.934	0.214	0.776	0.855
MP × E × 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 × 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

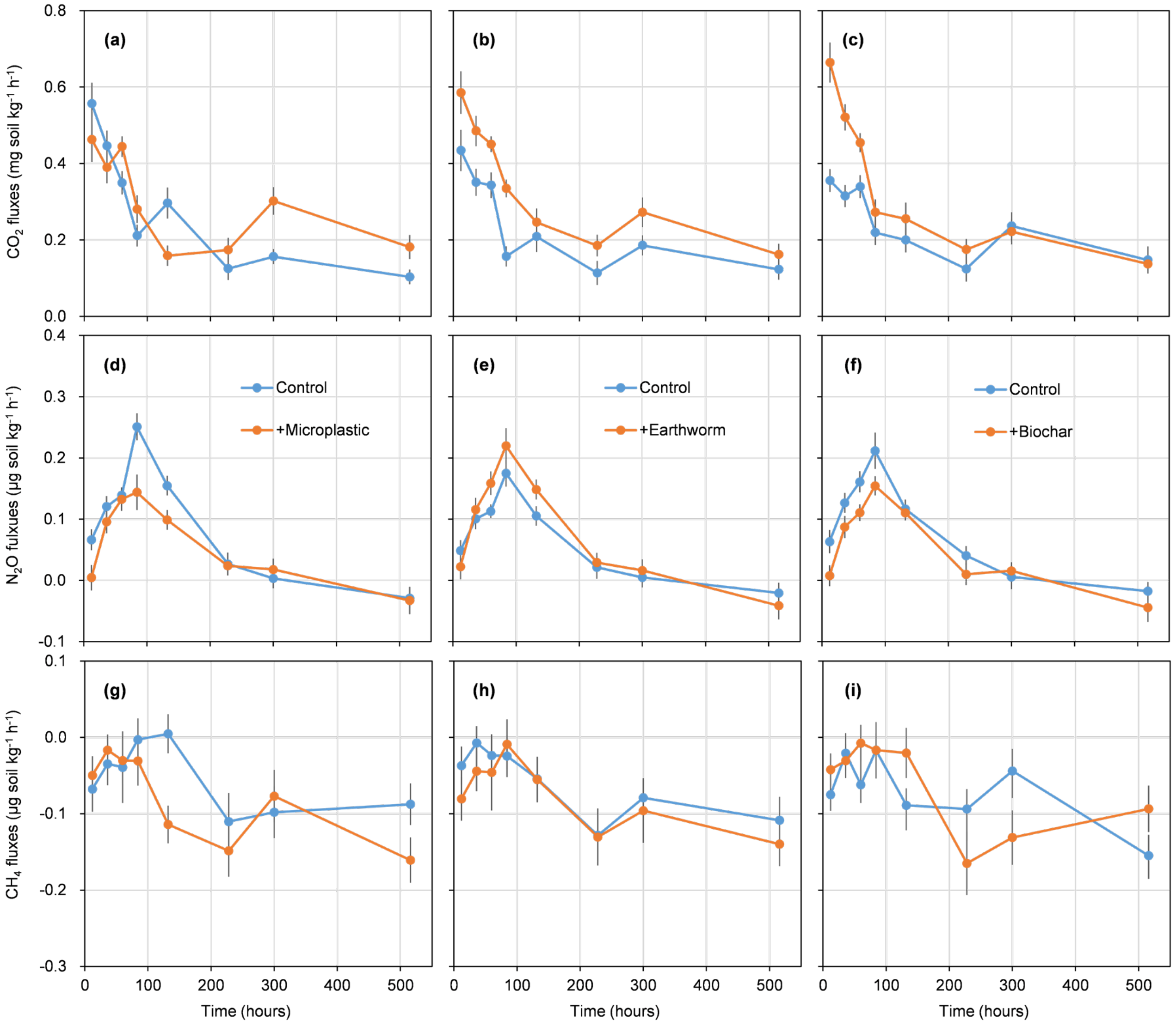

Fig. 1. Hourly CO_2 (a, b, and c), N_2O (d, e, and f), and CH_4 (g, h, and i) emission fluxes during the 21-day incubation. The error bars indicate the standard errors of means (±SE, n=16).

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

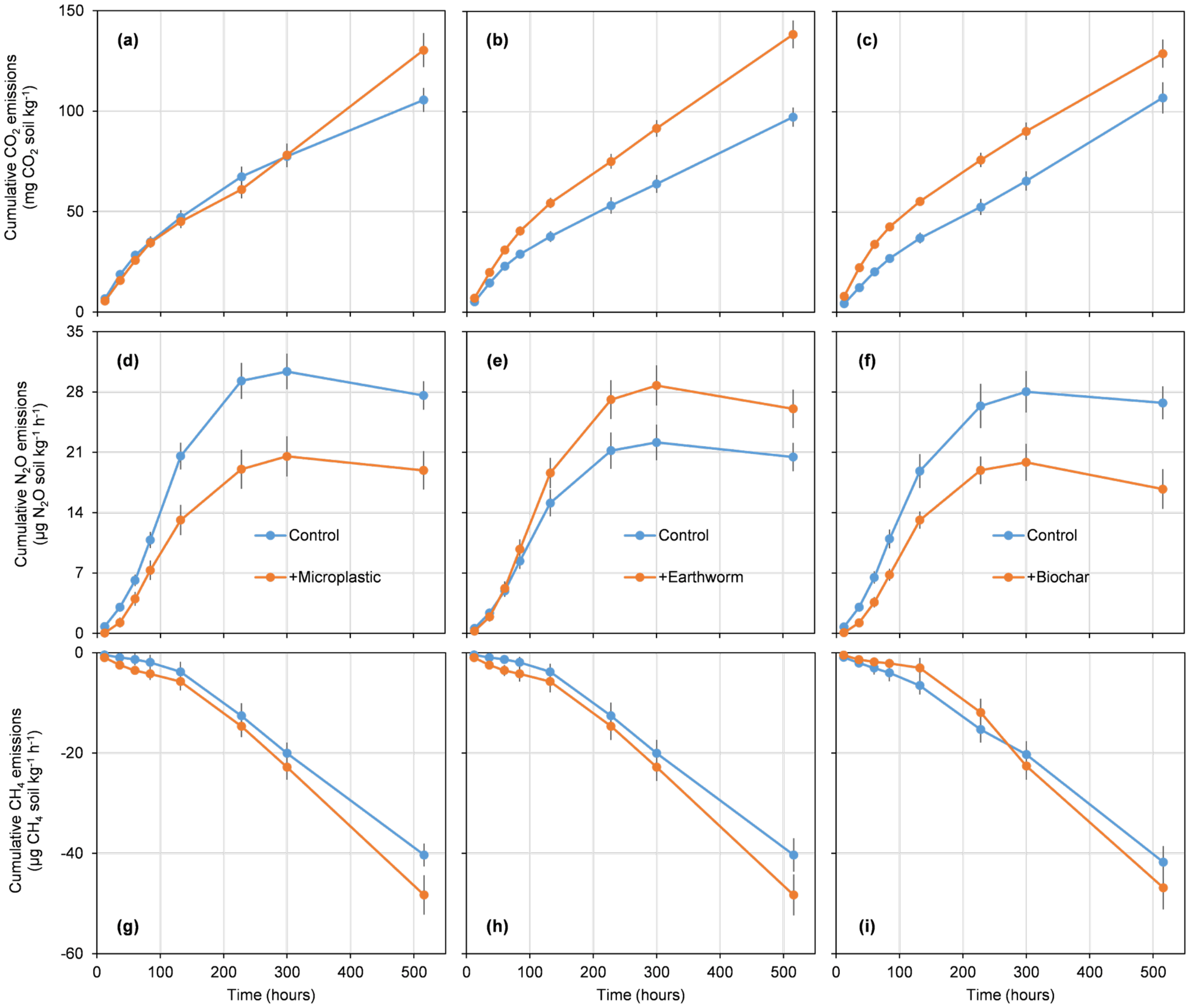

Fig. 2. Cumulative CO_2 (a, b, and c), N_2O (d, e, and f), and CH_4 (g, h, and i) emissions during the 21-day incubation. The error bars indicate the standard errors of means (±SE, n=16).

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 ± 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_2O (µg N_2O kg^{-1})		CH_4 (µg CH_4 kg^{-1})		GWP (mg $CO_{2\ (eq)}$ kg^{-1})	
Microplastic (MP)								
Control	105.57±5.98	b	27.60±1.94	a	-36.02±2.28	a	112.57±5.95	b
+MP	130.36±8.44	a	18.91±1.57	b	-52.60±3.95	b	134.20±8.51	a
Earthworm (E)								
Control	97.35±4.80	b	20.46±1.65	b	-40.33±3.34	a	102.08±4.51	b
+E	138.57±6.91	a	26.05±2.23	a	-48.29±4.09	a	154.02±5.03	a
Biochar (B)								
Control	106.93±7.81	b	26.74±1.90	a	-41.76±3.22	a	113.48±7.76	b
+B	129.00±7.08	a	19.77±1.87	b	-46.86±4.32	a	133.29±7.07	a
ANOVA	*p* 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 × E	**<0.05**†		0.467		0.634		**<0.05**†	
MP × B	0.68		0.785		0.923		0.707	
E × B	0.76		0.834		0.657		0.755	
MP × E × B	0.95		0.747		0.905		0.975	

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

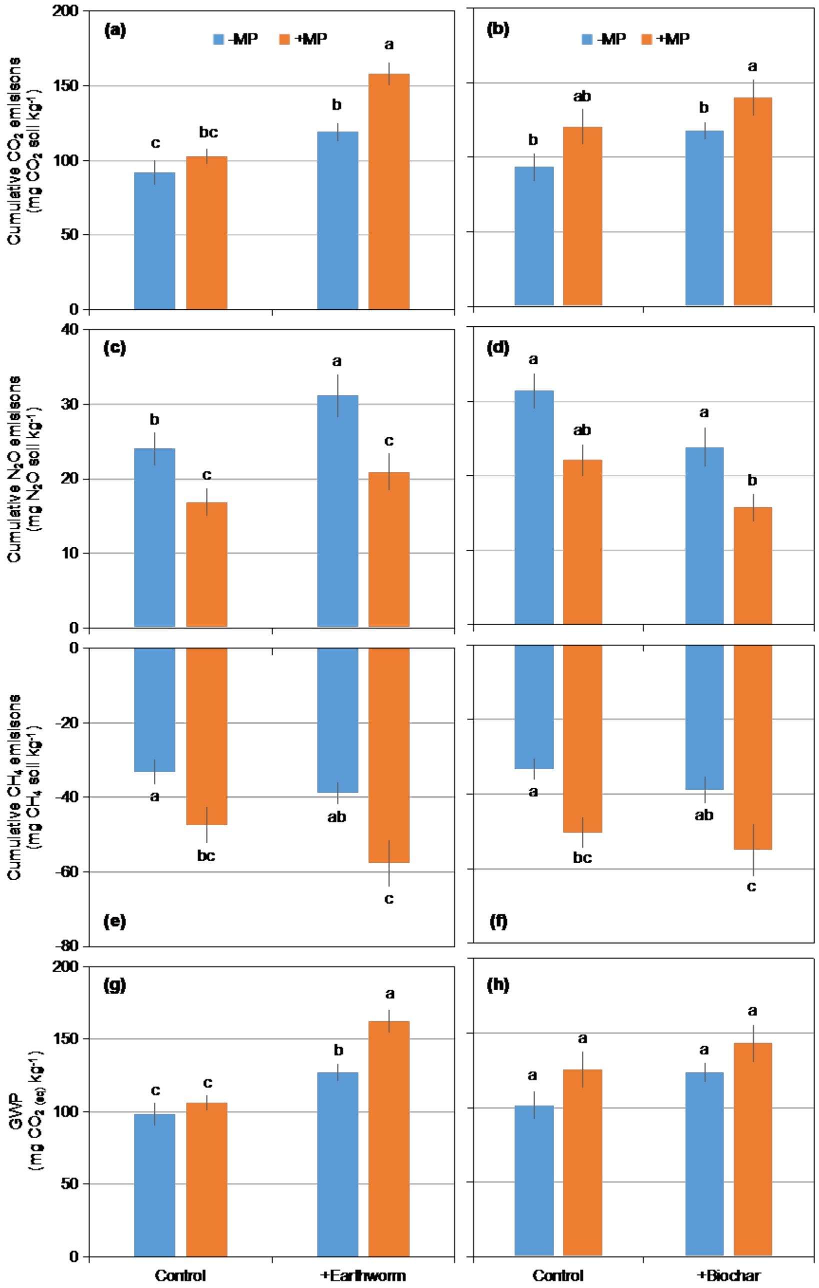

Fig. 2. Cumulative CO_2 (a, b, and c), N_2O (d, e, and f), and CH_4 (g, h, and i) emissions during the 21-day incubation. The error bars indicate the standard errors of means (±SE, n=16).

the interaction of MPs and earthworms (Table 4, Fig. 3). Specifically, when earthworms were absent, there was no significant difference in CO_2 emissions with or without MPs. However, the presence of earthworms significantly enhanced CO_2 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 CO_2 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 CO_2 emission. Similarly, earthworms significantly enhanced GWP by 27.7% when adding MPs (Fig. 3g). This interaction was closely associated with the combined contribution of CO_2, N_2O, and CH_4 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, NO_3^--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 CO_2 emissions and GWP. Furthermore, the changes in GHG emissions under different treatments exhibited variability. Specifically, MPs decreased N_2O emissions, while earthworms and biochar increased them. Conversely, CH_4 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 CO_2 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.

Acknowledgments

This work was financially supported by the Henan Province Higher Education Institutions Youth Talents Cultivation Plan (2023GGJS018).

Conflict of Interest

The authors declare no conflict of interest.

References

1. FENG Z., WANG L., WAN X., YANG J., PENG Q., LIANG T., WANG Y., ZHONG B., RINKLEBE J. Responses of soil greenhouse gas emissions to land use conversion and reversion - A global meta-analysis. Global Change Biology, **28** (22), 6665, **2022**.
2. IPCC (Intergovernmental Panel on Climate Change). Climate Change 2021: The Physical Science Basis. Cambridge University Press, Cambridge, **2021**.
3. LIU Y., TANG H., MUHAMMAD A., HUANG G. Emission mechanism and reduction countermeasures of agricultural greenhouse gases - a review. Greenhouse Gases: Science and Technology, **9** (2), 160, **2019**.
4. SHAKOOR A., SHAKOOR S., REHMAN A., ASHRAF F., ABDULLAH M., SHAHZAD S.M., FAROOQ T.H., ASHRAF M., MANZOOR M.A., ALTAF M.M., ALTAF M.A. Effect of animal manure, crop type, climate zone, and soil attributes on greenhouse gas emissions from agricultural soils - A global meta-analysis. Journal of Cleaner Production, **278**, 124019, **2021**.
5. ZHANG S., WANG J., YAN P., HAO X., XU B., WANG W., AURANGZEIB M. Non-biodegradable microplastics in soils: A brief review and challenge. Journal of Hazardous Materials, **409**, 124525, **2021**.
6. ZHANG B., YANG X., CHEN L., CHAO J., TENG J., WANG Q. Microplastics in soils: a review of possible sources, analytical methods and ecological impacts. Journal of Chemical Technology and Biotechnology, **95** (8), 2052, **2020**.
7. HUANG Y., LIU Q., JIA W., YAN C., WANG J. Agricultural plastic mulching as a source of microplastics in the terrestrial environment. Environmental Pollution, **260**, 114096, **2020**.
8. YAO Y., WANG L., PAN S., LI G., LIU H., XIU W., GONG L., ZHAO J., ZHANG G., YANG D. Can microplastics mediate soil properties, plant growth and carbon/nitrogen turnover in the terrestrial ecosystem? Ecosystem Health and Sustainability, **8** (1), 2133638, **2022**.
9. ZHANG Z., YANG Z., YUE H., XIAO M., GE T., LI Y., YU Y., YAO H. Discrepant impact of polyethylene microplastics on methane emissions from different paddy soils. Applied Soil Ecology, **181**, 104650, **2023**.
10. SU P., BU N., LIU X., SUN Q., WANG J., ZHANG X., XIANG T., CHU K., ZHANG Z., CAO X., LI Z. Stimulated soil CO_2 and CH_4 emissions by microplastics: A hierarchical perspective. Soil Biology and Biochemistry, **194**, 109425, **2024**.
11. YU Y., LI X., FENG Z., XIAO M., GE T., LI Y., YAO H. Polyethylene microplastics alter the microbial functional gene abundances and increase nitrous oxide emissions from paddy soils. Journal of Hazardous Materials, **432**, 128721, **2022**.
12. SU P., GAO C., ZHANG X., ZHANG D., LIU X., XIANG T., LUO Y., CHU K., ZHANG G., BU N., LI Z. Microplastics stimulated nitrous oxide emissions primarily through denitrification: A meta-analysis. Journal of Hazardous Materials, **445**, 130500, **2022**.
13. YU Y., LI X., FAN H., LI Y., YAO H. Dose effect of polyethylene microplastics on nitrous oxide emissions from paddy soils cultivated for different periods. Journal of Hazardous Materials, **453**, 131445, **2023**.
14. SHRESTHA R.K., JACINTHE P.A., LAL R., LORENZ K., SINGH M.P., DEMYAN S.M., REN W., LINDSEY L.E. Biochar as a negative emission technology: A synthesis of field research on greenhouse gas emissions. Journal of Environmental Quality, **52** (4), 769, **2023**.
15. SULTAN H., LI Y., AHMED W., YIXUE M., SHAH A., FAIZAN M., AHMAD A., ABBAS H.M.M., NIE L., KHAN M.N. Biochar and nano biochar: Enhancing salt resilience in plants and soil while mitigating greenhouse gas emissions: A comprehensive review. Journal of Environmental Management, **355**, 120448, **2024**.
16. NAN Q., HU S., QIN Y., WU W. Methane oxidation activity inhibition via high amount aged biochar application in paddy soil. Science of The Total Environment, **796**, 149050, **2021**.
17. WU Z., ZHANG X., DONG Y., LI B., XIONG Z. Biochar amendment reduced greenhouse gas intensities in the rice-wheat rotation system: six-year field observation and meta-analysis. Agricultural and Forest Meteorology, **278**, 107625, **2019**.
18. WANG L., GAO C., YANG K., SHENG Y., XU J., ZHAO Y., LOU J., SUN R., ZHU L. Effects of biochar aging in the soil on its mechanical property and performance

for soil CO_2 and N_2O emissions. Science of The Total Environment, **782**, 146824, **2021**.
19. JEFFERY S., VERHEIJEN F.G.A., KAMMANN C., ABALOS D. Biochar effects on methane emissions from soils: A meta-analysis. Soil Biology and Biochemistry, **101**, 251, **2016**.
20. CAI F., FENG Z., ZHU L. Effects of biochar on CH_4 emission with straw application on paddy soil. Journal of Soils and Sediments, **18** (2), 599, **2017**.
21. OO A.Z., SUDO S., AKIYAMA H., WIN K.T., SHIBATA A., YAMAMOTO A., SANO T., HIRONO Y. Effect of dolomite and biochar addition on N_2O and CO_2 emissions from acidic tea field soil. PLoS One, **13** (2), e0192235, **2018**.
22. LIU J., QIU H., WANG C., SHEN J., ZHANG W., CAI J., TANG H., WU J. Effects of biochar amendment on greenhouse gas emission in two paddy soils with different textures. Paddy and Water Environment, **19** (1), 87, **2020**.
23. JU H., YANG X., OSMAN R., GEISSEN V. Effects of microplastics and chlorpyrifos on earthworms (*Lumbricus terrestris*) and their biogenic transport in sandy soil. Environmental Pollution, **316**, 120483, **2023**.
24. GONG X., LI J., CHANG S., WU Q., AN Z., HUANG C., SUN X., LI S., WANG H. Cattle manure biochar and earthworm interactively affected CO_2 and N_2O emissions in agricultural and forest soils: Observation of a distinct difference. Frontiers of Environmental Science & Engineering, **16** (3), 39, **2021**.
25. FOREY O., SAUZE J., PIEL C., GRITTI E.S.,DEVIDAL S., FAEZ A., RAVEL O., NAHMANI J., ROUCH L., BLOUIN M., PÉRÈS G., CAPOWIEZ Y., ROY J., MILCU A. Earthworms do not increase greenhouse gas emissions (CO_2 and N_2O) in an ecotron experiment simulating a three-crop rotation system. Scientific Reports, **13**, 21920, **2023**.
26. GAO B., LI Y., ZHENG N., LIU C., REN H., YAO H. Interactive effects of microplastics, biochar, and earthworms on CO_2 and N_2O emissions and microbial functional genes in vegetable-growing soil. Environmental Research, **213**, 113728, **2022**.
27. ZAITSEV A.S., GORBUNOVA A.Y., KOROBUSHKIN D.I., DEGTYAREV M.I., ZHADOVA A.N., KOSTINA N.V., GONGALSKY K.B. The earthworm species Eisenia fetida modulates greenhouse gas release and carbon stabilization after rice straw amendment to a paddy soil. European Journal of Soil Biology, **89**, 39, **2018**.
28. NAMOI N., PELSTER D., ROSENSTOCK T.S., MWANGI L., KAMAU S., MUTUO P., BARRIOS E. Earthworms regulate ability of biochar to mitigate CO_2 and N_2O emissions from a tropical soil. Applied Soil Ecology, **140**, 57, **2019**.
29. GANAULT P., NAHMANI J., CAPOWIEZ Y., FROMIN N., SHIHAN A., BERTRAND I., BUATOIS B., MILCU A. Earthworms and plants can decrease soil greenhouse gases emissions by modulating soil moisture fluctuations and soil macroporosity in a mesocosm experiment. PLoS One, **2**, 19, **2023**.
30. LUO Y., ZANG H., YU Z., CHEN Z., GUNINA A., KUZYAKOV Y., XU J., ZHANG K., BROOKES P.C. Priming effects in biochar enriched soils using a three-source-partitioning approach: ^{14}C labelling and ^{13}C natural abundance. Soil Biology and Biochemistry, **106**, 28, **2017**.
31. ZHANG Y., MAI H., QIU Q., ZHU Y., LONG J., CHEN S., CHEN Y. The Responses of C, N, P and Stoichiometric Ratios to Biochar and Vermicompost Additions Differ from Alfalfa and a Mine Soil. Agriculture, **13** (10), 1954, **2023**.
32. ZHU X., CHANG L., LI J., LIU J., FENG L., WU D. Interactions between earthworms and mesofauna affect CO_2 and N_2O emissions from soils under long-term conservation tillage. Geoderma, **332**, 153, **2018**.
33. YANG W., YANG M., WEN H., JIAO Y. Global Warming Potential of CH_4 uptake and N_2O emissions in saline–alkaline soils. Atmospheric Environment, **191**, 172, **2018**.
34. RILLIG M.C., LEIFHEIT E., LEHMANN J. Microplastic effects on carbon cycling processes in soils. PLoS Biology, **19** (3), e3001130, **2021**.
35. DE SOUZA MACHADO A.A., LAU C.W., TILL J., KLOAS W., LEHMANN A., BECKER R., RILLIG M.C. Impacts of Microplastics on the Soil Biophysical Environment. Environmental Science & Technology, **52** (17), 9656, **2018**.
36. SHI J., WANG Z., PENG Y., FAN Z., ZHANG Z., WANG X., ZHU K., SHANG J., WANG J. Effects of Microplastics on Soil Carbon Mineralization: The Crucial Role of Oxygen Dynamics and Electron Transfer. Environmental Science & Technology, **57** (36), 13588, **2023**.
37. KAN Z., ZHOU J., LI F., SHETEIWY M.S., QI J., CHEN C., YANG H. Straw incorporation interacting with earthworms mitigates N_2O emissions from upland soil in a rice-wheat rotation system. Science of The Total Environment, **859**, 160338, **2023**.
38. HAN L., CHEN L., LI D., JI Y., FENG Y., FENG Y., YANG Z. Influence of polyethylene terephthalate microplastic and biochar co-existence on paddy soil bacterial community structure and greenhouse gas emission. Environmental Pollution, **292**, 118386, **2022**.
39. YAO Z., YAN G., MA L., WANG Y., ZHANG H., ZHENG X., WANG R., LIU C., WANG Y., ZHU B., ZHOU M., RAHIMI J., BUTTERBACH-BAHL K. Soil C/N ratio is the dominant control of annual N_2O fluxes from organic soils of natural and semi-natural ecosystems. Agricultural and Forest Meteorology, **327**, 109198, **2022**.
40. KLEMEDTSSON L., VON ARNOLD K., WESLIEN P., GUNDERSEN P. Soil CN ratio as a scalar parameter to predict nitrous oxide emissions. Global Change Biology, **11** (7), 1142, **2005**.
41. SUN X., TAO R., XU D., QU M., ZHENG M., ZHANG M., MEI Y. Role of polyamide microplastic in altering microbial consortium and carbon and nitrogen cycles in a simulated agricultural soil microcosm. Chemosphere, **312**, 137155, **2023**.
42. LI Z., TANG Z., SONG Z., CHEN W., TIAN D., TANG S., WANG X., WANG J., LIU W., WANG Y., LI J., JIANG L., LUO Y., NIU S. Variations and controlling factors of soil denitrification rate. Global Change Biology, **28** (6), 2133, **2022**.
43. LI Z., ZENG Z., SONG Z., TIAN D., HUANG X., NIE S., WANG J., JIANG L., LUO Y., CUI J., NIU S. Variance and main drivers of field nitrous oxide emissions: A global synthesis. Journal of Cleaner Production, **353**, 131686, **2022**.
44. ARAUJO P.I., PIÑEIRO-GUERRA J.M., YAHDJIAN L., ACRECHE M.M., ALVAREZ C., ALVAREZ C.R., COSTANTINI A., CHALCO VERA J., DE TELLERÍA J., DELLA CHIESA T., LEWCZUK N.A., PETRASEK M., PICCINETTI C., PICONE L., PORTELA S.I., POSSE G., SEIJO M., VIDELA C., PIÑEIRO G. Drivers of N_2O Emissions from Natural Forests and Grasslands Differ in Space and Time. Ecosystems, **24** (2), 335, **2020**.
45. LV M., FU S., SHAO Y., LIN Y., WU J., ZHANG W. Earthworm Pontoscolex corethrurus stimulated soil CO_2

emission by enhancing substrate availability rather than changing microbiota community structure. Science of The Total Environment, **717**, 137227, **2020**.

46. LUBBERS I.M., VAN GROENIGEN K.J., FONTE S.J., SIX J., BRUSSAARD L., VAN GROENIGEN J.W. Greenhouse-gas emissions from soils increased by earthworms. Nature Climate Change, **3** (3), 187, **2013**.
47. KAN Z.R., WEI Q., YANG R., LI Y., ZHOU J., QI J., LI F.M., YANG H. Arbuscular mycorrhizal fungi mitigate earthworm-induced N_2O emissions from upland soil in a rice-rotated wheat farming system. Applied Soil Ecology, **189**, 104981, **2023**.
48. KUIPER I., DE DEYN G.B., THAKUR M.P., VAN GROENIGEN J.W. Soil invertebrate fauna affect N_2O emissions from soil. Global Change Biology, **19** (9), 2814, **2013**.
49. LI Y., LIAO J., CHEN H.Y.H., ZOU X., DELGADO-BAQUERIZO M., NI J., REN T., XU H., RUAN H. Soil fauna alter the responses of greenhouse gas emissions to changes in water and nitrogen availability. Soil Biology and Biochemistry, **179**, 108990, **2023**.
50. MITRA P., KANEKO N. Impact of Aquatic Earthworms on Methane Emission Reduction from the Paddy Field Soil in Japan. Journal of Agricultural Science, **9** (10), 36, **2017**.
51. WALKIEWICZ A., KALINICHENKO K., KUBACZYŃSKI A., BRZEZIŃSKA M., BIEGANOWSKI A. Usage of biochar for mitigation of CO_2 emission and enhancement of CH_4 consumption in forest and orchard Haplic Luvisol (Siltic) soils. Applied Soil Ecology, **156**, 103711, **2020**.
52. BORCHARD N., SCHIRRMANN M., CAYUELA M.L., KAMMANN C., WRAGE-MÖNNIG N., ESTAVILLO J.M., FUERTES-MENDIZÁBAL T., SIGUA G., SPOKAS K., IPPOLITO J.A., NOVAK J. Biochar, soil and land-use interactions that reduce nitrate leaching and N_2O emissions: A meta-analysis. Science of The Total Environment, **651**, 2354, **2019**.
53. HE Y., ZHOU X., JIANG L., LI M., DU Z., ZHOU G., SHAO J., WANG X., XU Z., HOSSEINI BAI S., WALLACE H., XU C. Effects of biochar application on soil greenhouse gas fluxes: a meta-analysis. GCB Bioenergy, **9** (4), 743, **2016**.
54. CHEN C., WANG K., ZHU H. Meta-Analysis of N_2O Emissions as Affected by Biochar Amendment in Northern China. Water, Air, & Soil Pollution, **235** (4), 238, **2024**.
55. DONG W., WALKIEWICZ A., BIEGANOWSKI A., OENEMA O., NOSALEWICZ M., HE C., ZHANG Y., HU C. Biochar promotes the reduction of N_2O to N_2 and concurrently suppresses the production of N_2O in calcareous soil. Geoderma, **362**, 114091, **2020**.
56. FENG Z., ZHU L. Impact of biochar on soil N_2O emissions under different biochar-carbon/fertilizer-nitrogen ratios at a constant moisture condition on a silt loam soil. Science of The Total Environment, **584**, 776, **2017**.
57. RILLIG M.C., HOFFMANN M., LEHMANN A., LIANG Y., LÜCK M., AUGUSTIN J. Microplastic fibers affect dynamics and intensity of CO_2 and N_2O fluxes from soil differently. Microplastics and Nanoplastics, **1**, 3, **2021**.
58. NASER H.M., NAGATA O., SULTANA S., HATANO R. Carbon Sequestration and Contribution of CO_2, CH_4 and N_2O Fluxes to Global Warming Potential from Paddy-Fallow Fields on Mineral Soil Beneath Peat in Central Hokkaido, Japan. Agriculture, **10** (1), 6, **2019**.