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Biochar

ORIGINAL RESEARCH

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Biochar-microplastics interaction modulates soil nitrous oxide emissions and microbial communities

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Abstract

Biochar has been proposed as a soil amendment in vegetable fields, where the widespread use of plastic film leads to significant retention of microplastics (MPs) in the soil. However, the interactive effect of biochar and MPs on plant growth and soil functions remains poorly understood. Here, we conducted a pot experiment to examine the effects of biochar application in the presence of conventional and biodegradable microplastics (0.05% *w/w*) on the growth of coriander, soil nitrogen (N) cycling processes, and microbial communities. The results showed that biochar application increased aboveground biomass by increasing plant available N of NH_4^+, regardless of the presence of MPs. Biochar also significantly reduced soil nitrous oxide (N_2O) emissions by an average of 16% without MPs. However, when MPs were present, the effect of biochar on N_2O emissions was lessened depending on the MP type. Polylactic acid consistently reduced soil N_2O emissions and the abundance of N_2O production genes, irrespective of biochar application. Conversely, polyethylene without biochar reduced N_2O emissions primarily by inhibiting N-related functional genes responsible for nitrification and denitrification. This inhibitory effect was reversed when biochar was applied, leading to a 26% increase in N_2O emissions due to increased *nifH* and *nirK* gene abundance. Although biochar and MPs did not significantly alter microbial α-diversity, they altered the composition and structure of bacterial and fungal communities, linked to changes in soil N turnover. Our study underscores the critical role of MP type in assessing the effects of biochar on soil N cycling and N_2O emissions. Consequently, plastic pollution may complicate the ability of biochar to improve plant growth and soil functions, depending on the characteristics of the MPs.

Highlights

- Biochar significantly increased the aboveground biomass of coriander, independent of microplastics.
- Polylactic acid addition reduced N_2O emissions by inhibiting nitrification and denitrification with and without biochar addition.
- Inhibition of soil N_2O emissions by polyethylene was reversed to promotion with biochar addition due to the increasing abundance of *nifH* and *nirK*.
- Effects of microplastics on the diversity and composition of soil microbial communities were related to their type and biochar addition.

Keywords Microplastics, Biochar, Microbes, N_2O, Plant growth, Biodegradable plastic

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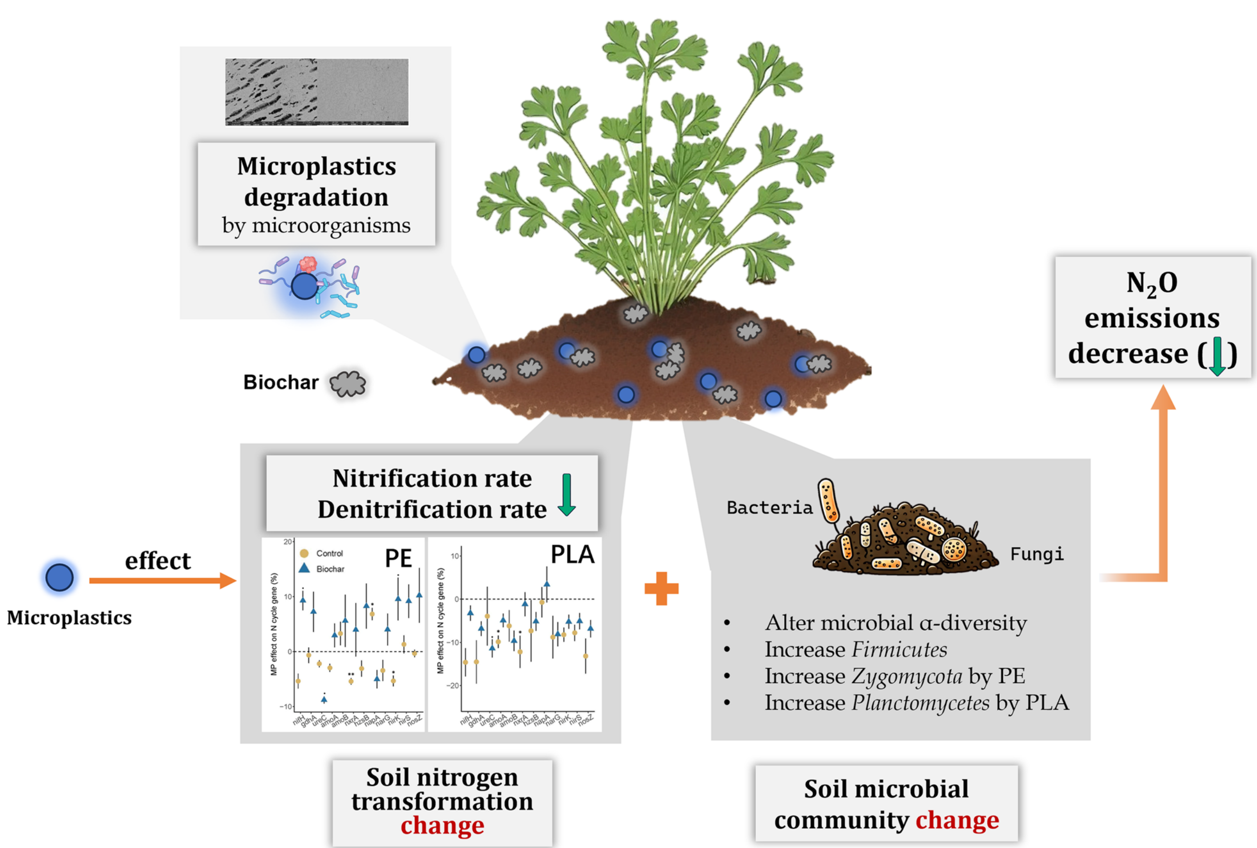
Graphical Abstract



1 Introduction

Over the past century, the widespread use of plastics has led to environmental microplastic pollution. Global plastic production exceeded 400 million tons in 2020 and is expected to double by 2035 (UNEP 2022), while only 9% of plastic waste is recycled (OECD 2022). As plastics persist in the environment and gradually degrade, they form MPs with particles smaller than 5 mm that accumulate in ecosystems. This issue is particularly prominent in agroecosystems, where plastics are applied through irrigation, mulching, and organic fertilizers (Serrano-Ruiz et al. 2021). In China alone, polyethylene (PE) mulching covered about 20 million hectares annually, highlighting the need to assess the potential impacts of MPs in agricultural soils (Palansooriya et al. 2023). Once MPs entered the soil, their waterproof and low-density characteristics caused changes in soil properties, such as reduced soil aeration, increased porosity, and altered carbon-to-nitrogen ratio (C/N) (Wang et al. 2022a). The accumulation of MPs in soil may threaten plant growth, microbial communities, and nutrient uptake by altering soil physicochemical properties(Wang et al. 2020).

The effects of MPs on soil structure and microbial functions varied depending on the type and concentration of MPs and experimental conditions. For instance, MP concentrations below 0.1% *w/w* (e.g., polystyrene PS, PE, and polypropylene PP) negatively affected plant growth by reducing soil fertility, as MPs clustered around root hairs, inhibiting water and nutrient uptake (Han et al. 2024; Lian et al. 2024). However, higher concentrations of PE-MPs (0.2% *w/w*) have shown either positive or no significant impact on the rhizosphere and plant performance (Han et al. 2024). MPs may also serve as habitats for pathogenic organisms, directly inhibiting plant growth through cytotoxic effects, though this is not always the case across studies (Chai et al. 2024). Different MPs varied in their C sources, influencing soil C decomposing and soil organic C (SOC) turnover, affecting soil microbial activity and community composition, ultimately altering soil N cycling and plant N uptake (Ge et al. 2021; Wang et al. 2024). Conventional and biodegradable plastics are two main categories of plastic. While conventional plastics are difficult to degrade, biodegradable plastics, such as polylactic acid (PLA), have been proposed as alternatives (Fan et al. 2022), which can theoretically break down into CO_2 and water through microbial actions (Moshood et al. 2022). Biodegradable MPs, such as PBAT-PLA (5% *w/w*), may cause different changes in microbial activity and N transformation compared to conventional MPs (PE and PS) after incubation (Hu et al. 2023; Wang et al. 2023). For example, PE has no significant effect on nitrification functional genes like *amo*A (Gao et al. 2021; Yu et al. 2022), while PLA reduced the *amo*A gene in the short term but increased it over time (Seeley et al. 2020). Consequently, the presence of MPs in soil and their impact on soil properties and microbial communities can have significant implications for plant growth and N cycling (Khalid et al. 2020; Wang et al. 2020), although a comprehensive understanding remains lacking.

Biochar has been recognized as a promising measure for improving soil quality and C sequestration (Joseph et al. 2021). By altering soil properties such as soil pH, porosity, and hydraulicity, biochar can contribute to the shift in soil water retention, microbial communities, and crop yields (Castellini et al. 2015; Sheng and Zhu 2018; Tanure et al. 2019). In addition, biochar reduced nitrous oxide (N_2O) emissions by modulating the expression of N-associated functional genes, which is supported by the 'electron shuttle' of biochar itself (Joseph et al. 2021). Biochar may also interact with MPs in the soil, influencing soil functions and greenhouse gas emissions. The interactive effect of biochar and MPs on N cycling varied, depending mainly on functional genes. For example, in soil incubation experiments, the reduction in N_2O emissions by PE addition was linked to a decrease in the *nirK* gene, while biochar application increased the *nirS* gene and thus stimulated N_2O emissions (Li et al. 2022b). Similarly, in plant-soil systems, the decrease in N_2O emissions by biochar application was reversed in PET-contaminated soil due to decreased *nosZ* gene abundance (Han et al. 2022). Plant growth also played a role in modifying the combined effects of biochar and MPs on soil N transformation. For instance, reduced NH_3 emissions under MPs (PE, PET, and PAN) with biochar application were attributed to increased NH_4^+ absorption by plant roots (Feng et al. 2022b). Since soil N dynamics are closely linked to soil C content, alterations in the N cycle in PE and biochar treatments might be attributed to reduced CO_2 emissions and lower SOM mineralization (Chen et al. 2024). However, the co-mechanisms influencing plant growth, soil properties, microbial communities, and greenhouse gas emissions from the co-application of biochar and MPs have not been well elucidated.

In this study, we conducted a pot experiment to explore the interactive effects of biochar and MPs in a soil–plant system. We used coriander as the model plant due to its widespread use as a seasoning worldwide. We examined how biochar, in combination with conventional (PE) and biodegradable (PLA) MP, would affect coriander growth, soil N_2O emissions, and microbial communities. We hypothesized that the impact of biochar application on promoting plant growth, improving soil properties, and reducing N_2O emissions may be influenced by adding MPs. These effects may vary depending on the type of MPs, given the differential availability of polymer C to soil microorganisms.

2 Materials and methods

2.1 Experimental design

A pot experiment was conducted in the greenhouse at Nanjing Agricultural University from July to October 2022. The soil, classified as Fluvisols (FAO, 1995), was collected from the top layer (0–10 cm) of a vegetable field in Xinhua City, Jiangsu province, eastern China (32°93'N, 119°82'E). Basic soil physiochemical properties include a pH of 4.86 (1:2.5, soil-to-water) and total N of 1.75 g kg^{-1}. Notably, no history of plastic application was found in the field. Before the initial experiment, the soil was sieved and homogenized using a 4 mm mesh size. Each pot (4 L, 13 in diameter, 11.5 cm deep) was filled with 1.2 kg of air-dried soil.

We purchased transparent PE mulching film (thickness 0.01 mm) and PLA drinking straws from Zhenhao Industrial Co., Ltd (Taizhou, China). PE is a commonly used agricultural mulching film in China, while PLA, a bio-based plastic, is considered a promising alternative to replace artificial petroleum-based plastics (Trivedi et al. 2023). The plastics were sterilized using 70% ethanol, washed with deionized water, and air-dried before being cut into fragments (2 mm × 1 mm) to mimic common sizes found in the soil (Wang et al. 2022a). The peanut-shell biochar was purchased from the Sanli New Energy Co., Ltd (Shangqiu, Henan, China). The biochar was produced through pyrolysis and thermal decomposition at 500 °C, with an initial pH of 9.4 and a total C content of 467 g kg^{-1}, respectively.

To examine the effects of plastic exposure, biochar application, and their interactions, an 80-day two-factorial experiment of six treatments with four replicates was designed (Fig. S1). PE and PLA were used in the concentration of 0.05% (*w/w*), both with and without biochar (BC) amendment (Control, Biochar, BC + PE and BC + PLA). Coriander (*Coriandrum sativum* L.) was selected as the model leafy vegetable commonly cultivated in China. The seeds were obtained from Yonghong Seeds Co., Ltd (Hebei, China). For each treatment, the biochar and microplastic fragments were evenly mixed into the dry soil of each pot before sowing. Two weeks post-sowing, the seedlings were thinned to four per pot. To ensure soil nutrients for plant growth, a compound fertilizer (N: P: K = 15:15:15) was applied at a rate of 50 kg N ha^{-1}. This fertilizer was dissolved in 50 mL of distilled water and applied to the soil surface one week after thinning. Pots were watered every two days by spraying 200 mL of distilled water evenly onto the soil surface throughout the experiment. All pots were randomly placed within the greenhouse and repositioned weekly to ensure uniform environmental conditions.

2.2 Measurement of plant and soil properties

Upon completion of the experiment, the plants were harvested and separated into above-ground parts (shoots and leaves) and underground parts (roots). These plant parts were dried at 60 °C for 72 h and weighed. Microplastic samples were picked up manually from the pots using tweezers, washed with sterile water, dried, and stored at 4 °C for subsequent scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) analysis. SEM images and FTIR characterization of the two types of MPs are shown in Fig. S2, and the relevant description is shown in Text S1. Soil samples were passed through a 2 mm mesh, homogenized, and divided into two portions. One portion was stored at 4 °C for soil physicochemical analyses, while the other was kept at − 80 °C for DNA extraction within a week.

The soil moisture content was determined gravimetrically by drying soil in an oven at 105 °C for 24 h. Soil pH was measured in the soil-to-distilled water mixture of 1: 2.5 (*w/v*). The concentrations of ammonium N (NH_4^+–N) and nitrate N (NO_3^-–N) were determined from soil extracts of 2 M KCl using colorimetric methods on a microplate reader (BioTek, USA). The soil DOC content was quantified from water extract using a Multi N/C 2100 TOC analyzer (AnalytikJena, Jena, Germany). To determine net N mineralization and nitrification rates, soil samples were aerobically incubated at 25 °C in the dark (Hart et al. 1994). Ammonia-oxidizing archaea (AOA) and bacteria (AOB) determine the first and rate-limiting steps in the nitrification process. The rates of potential nitrification (PNR) and denitrification (PDR) were determined using the shaken-slurry method (Hart et al. 1994) and then the modified acetylene inhibition technique (Norton and Stark 2011) to measure autotrophic, AOA-driven and AOB-driven nitrification, respectively.

2.3 Measurement of N_2O emissions

Over the experimental period, N_2O fluxes were monitored by the static chamber-gas chromatograph method (de Klein and Harvey 2012). Airtight chambers were placed over the pots to collect gas samples. The headspace gas was sampled three times at 20-min intervals using a syringe connected to a three-way valve. The gas samples were analyzed by gas chromatograph (Agilent 7890B, Agilent, USA) within a few hours of collection. Fluxes were adjusted for sampling temperature, chamber volume, and chamber cross-sectional area and then calculated via linear regression using all time points sampled (Clayton et al. 1994). To calculate the average N_2O emissions over the entire experiment, cumulative emissions were estimated using the trapezoid rule, assuming constant flux rates per day, then divided by the experiment days.

2.4 DNA extraction and quantitative PCR assays

DNA was extracted from 0.25 g of fresh soil samples using the Qiagen DNeasy PowerSoil Kit (Mo Bio, USA) following the manufacturer's protocols. The concentration and quality of genomic DNA were assessed using a Nanodrop ND-100 spectrophotometer (Thermo Scientific, USA). To determine the copy number of the genes involved in N cycling, real-time quantitative PCR was performed on each DNA sample with three analytical replicates by HT-qPCR QMEC (Zheng et al. 2018). The PCR conditions and primers for these genes are described in Table S1.

2.5 High-throughput amplicon sequencing and bioinformatic analysis

The extracted DNA samples were subjected to the high-throughput amplicon sequencing of bacterial and fungal communities. The primers of 515F/806R were used to amplify the bacterial 16S rRNA in the V4–V5 hypervariable region to analyze the bacterial community composition. The primers of ITS1F/ITS2R were applied to amplify the fungal ITS gene for the fungal community. The PCR products were purified with AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The samples were sequenced on an Illumina NovaSeq platform at LC-Biological Technology Co. Ltd to generate paired-end reads assigned to samples based on their unique barcode. Paired-end reads were merged using FLASH. The raw reads were filtered under specific conditions for high-quality clean tags according to the fqtrim (v0.94). Chimeric sequences were filtered using Vsearch software (v2.3.4). Following dereplication, quality control and identification of amplicon sequence variants were conducted using DADA2. Raw sequences were deposited in the NCBI database under accession number PRJNA1117047.

2.6 Statistical analysis

All statistical analyses were conducted in R 4.0.3. Before analysis, the data were checked for both normality (using Shapiro–Wilk's test) and homogeneity of variance (using Levene's test). Some data were either square-root or logarithmically transformed to meet the assumption. A two-way analysis of variance (ANOVA) was performed to assess the effects of microplastic exposure and biochar application and their interactions on N_2O flux, soil physicochemical properties, plant biomass, and functional genes. When necessary, the function *varIdent* was applied to account for heterogeneity in the treatment. Multiple comparisons were made using the least significant difference (LSD) method. Effects were considered statistically significant at a level of 0.05.

For the microbial analysis, the α-diversity (observed bacterial and fungal communities for all treatments) was presented by Ace, Shannon, Richness, and Chao1 metrics. The feature abundance was normalized using the relative abundance of each sample according to the SILVA (release 138) classifier. MP exposure and biochar application effects on observed species richness were evaluated using two-way ANOVA. To visualize and determine the effects of microplastic exposure and biochar application on community dissimilarity, an unconstrained principal coordinates analysis (PCoA) on Bray–Curtis dissimilarities was applied using the vegan package to depict the soil microbial beta diversity. A two-group permutational multivariate analysis of variance (PERMANOVA) was carried out using the *adonis* function with 10^4 permutations to assess the statistical significance of the observed differences. Structural equation modeling (SEM) analysis grouped by MP types was used to quantify and determine the hypothetical relationships between various factors, including soil properties, plant growth, N functional genes, bacterial diversity, N processes, and N_2O emissions. SEM was conducted by the "*piecewiseSEM*" package in R version 4.0.2 (Lefcheck and Freckleton 2015). The experimental indicators of the SEM building process are presented in Supporting Information. Nonmetric multidimensional scaling (NMDS) ordinations were calculated to identify differences in bacterial community composition and applied in SEM constructions. The best-fit model was determined using a Fisher's C test, *p*-values, and degrees of freedom (df). A distance-based redundancy analysis (db-RDA) was performed to analyze the relationship between the microbial and fungal community structure and soil physicochemical characteristics in response to different MP types. Statistically significant was determined at $P < 0.05$, with $0.05 < P < 0.1$ considered marginally significant.

3 Results

3.1 Plant and soil properties

Biochar addition significantly increased aboveground biomass by 26.9% ($P < 0.001$), while the presence of MPs had no significant effect on plant growth (Table 1). At the end of the experiment, applying biochar and MPs reduced soil moisture by 23.8% and 21.4%, respectively ($P < 0.05$, Table 1). The addition of MPs resulted in a decrease in soil pH ($P = 0.012$). The effect of biochar addition on soil EC was influenced by MP, with a significant decrease observed under PE application and an increase under PLA application in the LSD test. PLA notably enhanced NH_4^+ in soil by 15.2% without biochar addition. The effect of microplastic addition on soil NO_3^- and DOC depended on the presence of biochar. While applying biochar and MPs (averaging the results of PE and PLA treatments) individually reduced soil NO_3^- by 82.0% and 66.0%, respectively, the co-application of both (averaging the results of BC + PE and BC + PLA treatments) led to a threefold increase ($P < 0.001$). The significant decrease in DOC due to microplastic application was intensified under biochar application ($P < 0.001$).

3.2 N_2O, abundances of N-cycling functional genes, and nitrification process

Throughout the experiment, N_2O emission fluxes from all treatments exhibited similar emission patterns (Fig. 1a). Biochar and MPs (averaging the results of PE and PLA treatments) significantly reduced the average N_2O emissions by 21.7% and 25.3%, respectively ($P < 0.001$; Table 1). The negative effect of biochar on N_2O emissions was depressed by MP addition ($P < 0.001$), especially in the PE-treated soil, where the reduction was alleviated by 41.4%. Biochar application significantly decreased potential nitrification rates (PNR) and alleviated the decline in potential denitrification rates (PDR) induced by MPs, even leading to an increase with PE application. MPs and biochar exposure influenced N-cycle pathways through changes in functional genes (Fig. 1b and c). Besides the *hzs*B, *nxr*A, *nar*G, and *ure*C genes, microplastic and biochar applications significantly affected the abundance of N-cycle functional genes. Biochar exerted a marked negative effect on nitrification (*amo*A and *amo*B) and denitrification (*nir*S) processes ($P = 0.03$, 0.01 and 0.02). However, the impact of MPs on N functional genes varied depending on the type. The reduction in the relative abundance of *nif*H, *gdh*A, *amo*A, *nxr*A, *hzs*B, *nar*G,

Table 1 Effects of microplastics and biochar addition on soil properties, plant biomass, and N processes

	Without biochar			With biochar			ANOVA		
	Control	PE	PLA	Control	PE	PLA	Microplastics	Biochar	Interaction
Moisture ($g\ g^{-1}$)	0.21 ± 0.03^{a}	0.15 ± 0.00^{b}	0.18 ± 0.00^{ab}	0.16 ± 0.01^{b}	0.11 ± 0.01^{c}	0.15 ± 0.00^{b}	**7.71***	**12.09***	0.16
EC ($\mu S\ cm^{-1}$)	439.20 ± 6.40^{bc}	524.80 ± 31.20^{ab}	379.80 ± 39.90^{c}	362.10 ± 6.00^{c}	409.70 ± 8.10^{c}	541.00 ± 58.30^{a}	2.56	0.15	**10.67*****
pH	5.04 ± 0.04^{a}	4.97 ± 0.04^{ab}	4.83 ± 0.02^{b}	4.98 ± 0.03^{ab}	5.00 ± 0.09^{ab}	4.83 ± 0.08^{b}	**5.74***	0.11	0.32
NH_4^+ (mg N kg^{-1})	3.88 ± 0.05^{b}	4.23 ± 0.20^{ab}	4.47 ± 0.37^{a}	4.18 ± 0.03^{ab}	3.81 ± 0.08^{b}	4.65 ± 0.11^{a}	**5.53***	0.02	2.22
NO_3^- (mg N kg^{-1})	18.49 ± 0.34^{a}	3.67 ± 0.53^{c}	2.61 ± 0.49^{c}	3.32 ± 0.73^{c}	9.49 ± 1.68^{b}	10.63 ± 0.57^{b}	**17.13*****	0.41	**113.63*****
DOC (mg kg^{-1})	157.6 ± 6.00^{b}	143.50 ± 5.30^{bc}	140.20 ± 8.60^{bc}	191.80 ± 13.50^{a}	105.90 ± 2.10^{d}	132.10 ± 3.50^{c}	**24.59*****	0.39	**11.64*****
AGB biomass (g pot^{-1})	11.67 ± 1.14^{c}	13.80 ± 0.54^{bc}	11.51 ± 0.99^{c}	15.96 ± 1.17^{ab}	16.71 ± 0.35^{ab}	17.98 ± 1.55^{a}	1.00	**29.05*****	1.50
Root biomass (g pot^{-1})	0.030 ± 0.01^{ab}	0.03 ± 0.01^{ab}	0.02 ± 0.00^{b}	0.03 ± 0.00^{ab}	0.03 ± 0.00^{a}	0.04 ± 0.01^{a}	0.11	**7.63***	1.39
N_2O emissions (μg $kg^{-1}\ d^{-1}$)	56.33 ± 0.23^{a}	38.60 ± 0.53^{d}	45.56 ± 0.87^{b}	44.09 ± 0.84^{b}	46.19 ± 0.50^{b}	41.07 ± 0.99^{c}	**72.30*****	**27.56*****	**98.96*****
PNR (ug $kg^{-1}\ h^{-1}$)	0.18 ± 0.01^{a}	0.13 ± 0.02^{b}	0.12 ± 0.010^{b}	0.09 ± 0.00^{c}	0.11 ± 0.01^{bc}	0.12 ± 0.01^{bc}	1.29	**23.05*****	**9.02****
PDR (ug $kg^{-1}\ h^{-1}$)	18.13 ± 0.21^{a}	16.31 ± 0.53^{b}	10.09 ± 0.53^{d}	12.66 ± 0.39^{c}	17.46 ± 0.85^{ab}	12.64 ± 0.78^{c}	**46.92*****	1.49	**26.30*****

Values are means ± SE ($n=4$). $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$. Different superscript letters indicate significant differences between treatments at $P<0.05$. *EC* Electrical Conductivity, *AGB* Aboveground biomass, *PNR* Potential nitrification rate, *PDR* Potential denitrification rate. Different lowercase letters in the same row represent significant differences for multiple comparisons

*nir*K, and *nos*Z due to PE treatments was reversed upon biochar addition in soil (Fig. 1b and c). In contrast, the increase in the relative abundance of *amo*B and *nir*S induced by PE application was independent of biochar. In the case of PLA, biochar no longer played a determining role in the abundance of N functional genes. PLA application significantly reduced the relative abundance of *nif*H, *gdh*A, *ure*C, *amo*A, *amo*B, *nxr*A, *nar*G, *nir*K, *nir*S, and *nos*Z. MPs and their interactions with biochar significantly affected the relative abundance of the *nirK* gene. Biochar reversed the inhibitory effect of microplastic addition on the *nir*K gene in PE-treated soil but alleviated the negative impact on PLA-treated soil.

PE application significantly reduced the nitrification rate (NR), alleviating this effect by biochar addition (Fig. 2a). The inhibitory effects of biochar on the NR and AOB-driven nitrification rate were alleviated considerably in the presence of MPs ($P=0.01$ and 0.04), especially in response to PLA treatment (Fig. 2b). The AOA-driven nitrification rate in PLA treatments was about 5.5 times higher than that in the control and PE ($P<0.001$) and was significantly reduced by 77.4% under biochar application (Fig. 2c). The rate of autotrophic nitrification (AN) was reduced considerably by biochar and PE by 70.0% and 28.8%, respectively ($P<0.05$; Fig. 2b). The ratio of AOA to AOB in AN significantly enhanced following biochar application (Fig. 2e and f).

3.3 Microbial community structure and diversity

After merging and filtering the raw data of 16S and ITS reads, 22,778 and 39,616 high-quality sequences were obtained, respectively. The α-diversity of bacterial and fungal communities did not show significant differences, as indicated by Richness, Shannon, ACE, and Chao1 indices (Fig. 3a and c). The differences in the soil microbiota between treatments were detectable at the phylum level (Fig. 3b and d). *Actinobacteria*, *Proteobacteria,* and *Chloroflex* were identified as the dominant phyla of bacteria, accounting for over 60% of the total species in soil (Fig. 3b). The relative abundance of *Firmicutes* increased with MPs and biochar application. *Zygomycota* increased with PE application under biochar amendment. *Planctomycetes* decreased in response to PLA treatment (Fig. 3b). For fungi, the proportion of *Ascomycota* exceeded 60% and increased with biochar application (Fig. 3d).

To compare the responses of microbial communities among different treatments, β-diversity was assessed using PCoA with Bray Curtis dissimilarity metrics (Fig. 4a). The first two axes of bacterial community composition explained 19.1% and 15.5% of the total variation,

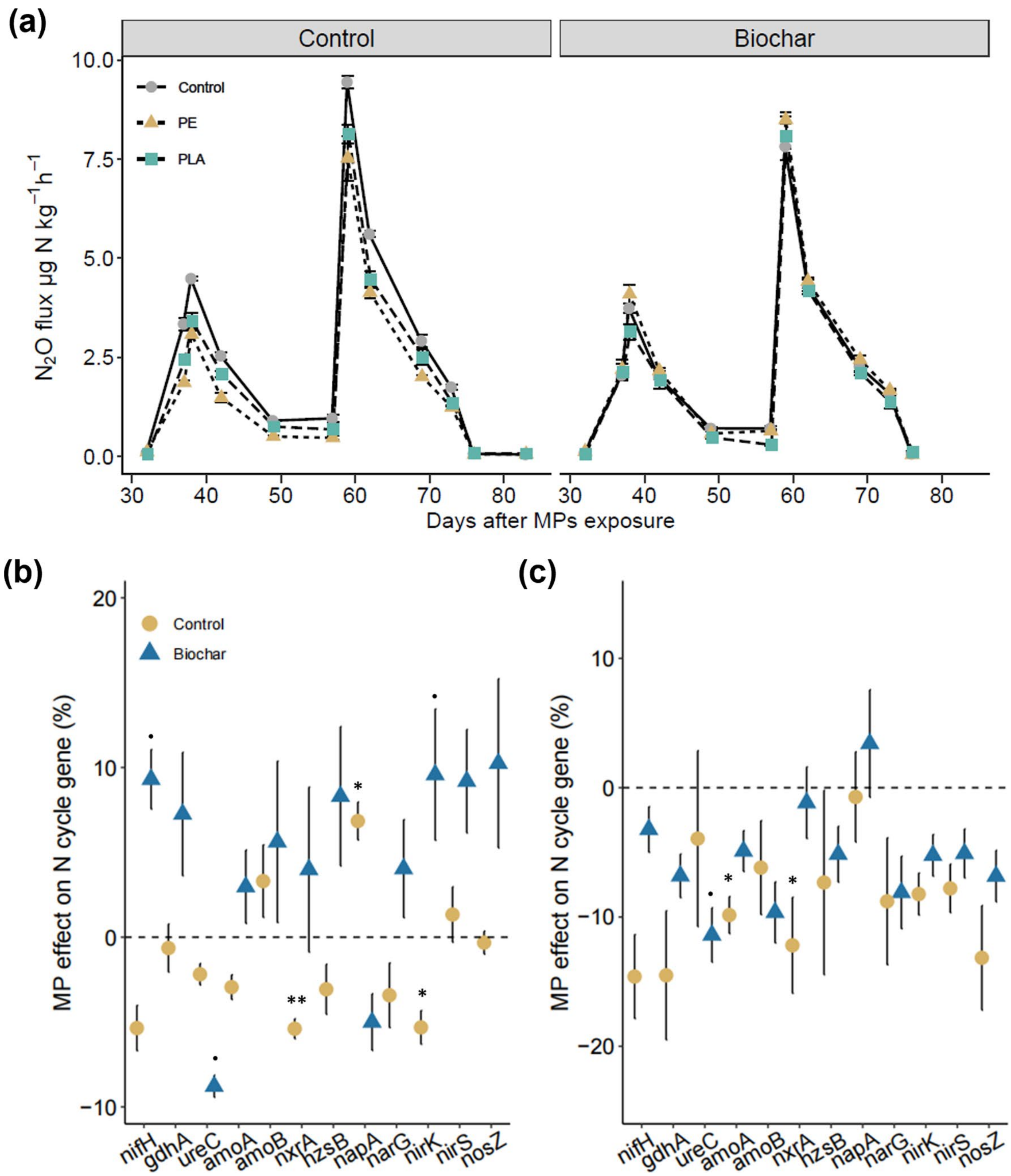


Fig. 1 Dynamics of N_2O fluxes from treatments during the experimental cycle (**a**), and percent changes in the relative abundance of N cycle genes in PE (**b**) and PLA treatments (**c**) with and without biochar application. Values are means ± standard error (SE, $n = 4$). Genes with significant differences between treatments with and without microplastic addition are indicated by asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$)

while those for fungi accounted for 30.8% and 18.6%, respectively. The PERMANOVA results indicated that the effects of biochar and MPs had significant impacts on the β-diversity of both bacterial ($P = 0.03$ to < 0.001) and fungal communities ($P = 0.01$ to < 0.001). However, only bacteria shifted significantly due to the interactions of MPs and biochar ($P = 0.005$). In particular, the bacterial community composition under PE treatment exhibited a distinct separation along axis 1 in the presence of biochar, in contrast to the results observed in PLA treatments. Regarding fungal communities, biochar application led to a clear separation along axis 1, whereas different MP types resulted in subtle clustering along axis 2 compared to the control group (Fig. 4c). As the PCoA

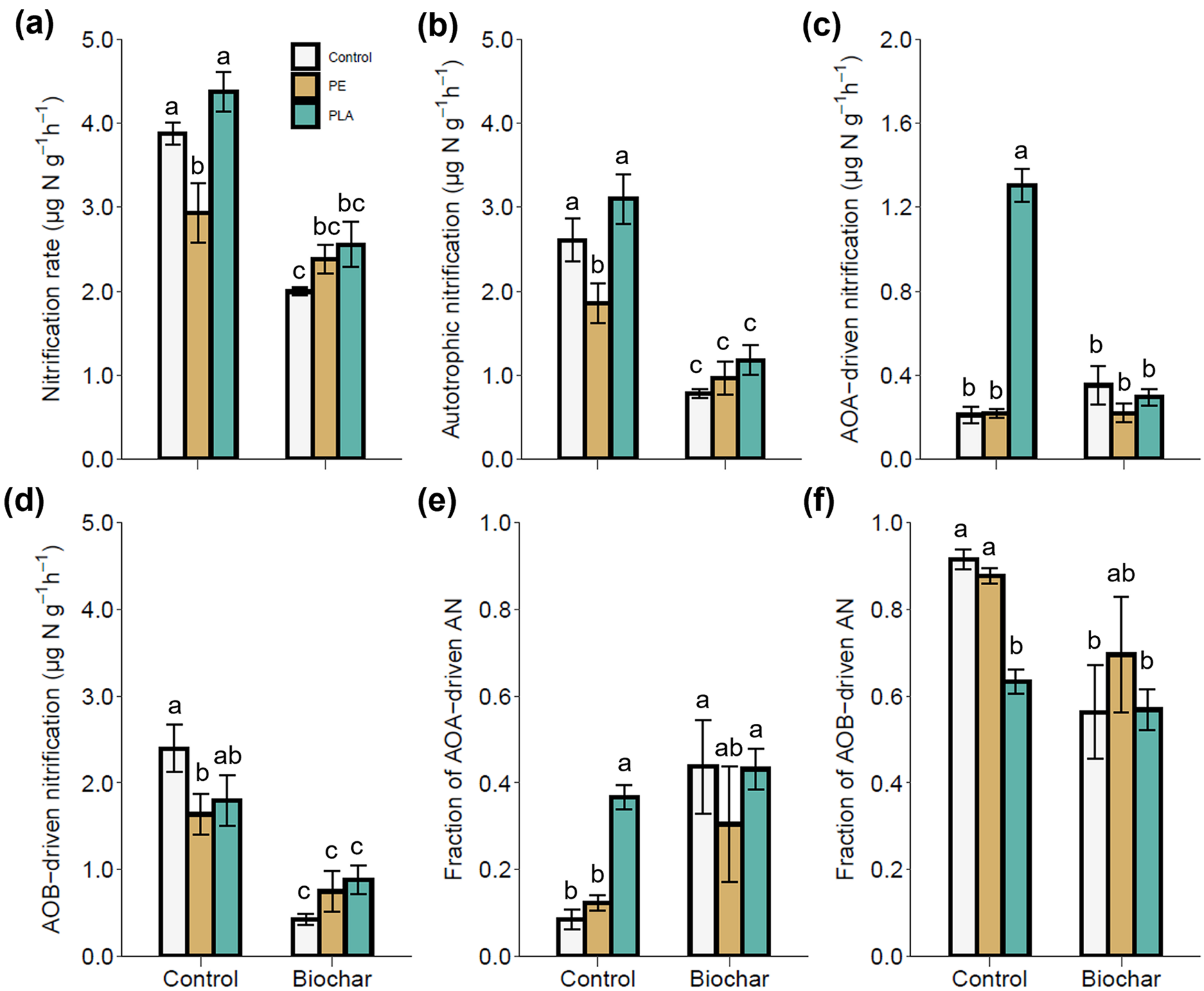


Fig. 2 Effects of biochar amendment and microplastic addition on the rates of nitrification (**a**), autotrophic nitrification (AN, **b**), AOA-driven nitrification (**c**), AOB-driven nitrification (**d**), and the fraction of AOA-driven (**e**) and AOB-driven (**f**) in autotrophic nitrification. Values are means ± SE ($n = 4$). Different lowercase letters indicate significant differences between treatments at $P < 0.05$

plot showed significant variation between PE and PLA, db-RDA analysis was grouped by the MP types to reveal the relationship between the microbial composition and the selected environmental factors. The bacterial taxonomic composition in PLA-treated soil exhibited strong correlations with the soil NO_3^-, PNR, PDR ($P < 0.05$) and N_2O emissions ($P = 0.07$; Fig. 4b). Conversely, PE application demonstrated correlations with soil EC, PDR, and DOC ($P < 0.05$) for bacteria. For fungi, PE treatment was associated with significant correlations with soil moisture, EC, NO_3^-, PNR, N_2O ($P < 0.1$), and NH_4^+ ($P < 0.1$). In contrast, pH ($P = 0.04$) and DOC ($P = 0.01$) were the two main drivers of the variation in fungal community composition under PLA application (Fig. 4d).

We constructed structural equation models to examine the relationships among various factors, including soil properties, plant growth, N functional genes, bacterial diversity, N processes, and N_2O emissions (Fig. 5). Our analysis focused on different types of MPs. The piecewiseSEMs of PE + BC (Fisher's C = 35.71, $P = 0.218$) and PLA + BC (Fisher's C = 35.22, $P = 0.235$) indicated that the interpretation rates of all factors for N_2O emissions were 96% and 95%, respectively. Adding MPs positively impacted soil physiochemical properties and bacterial composition, particularly significant for the type of PE (path coefficients = 0.5; $P < 0.05$). In soils treated with PE and biochar, biochar showed significant positive associations with plant growth, bacterial communities, and N processes (path coefficients = 0.68, 0.435, and 0.626, $P < 0.05$, Fig. 5a) while exhibiting a negative relationship with N_2O emissions (path coefficient = −0.241, $P < 0.05$). Furthermore, N_2O emissions were significantly influenced by soil properties and bacterial communities (path coefficients = 0.5). In soils treated with PLA and biochar,

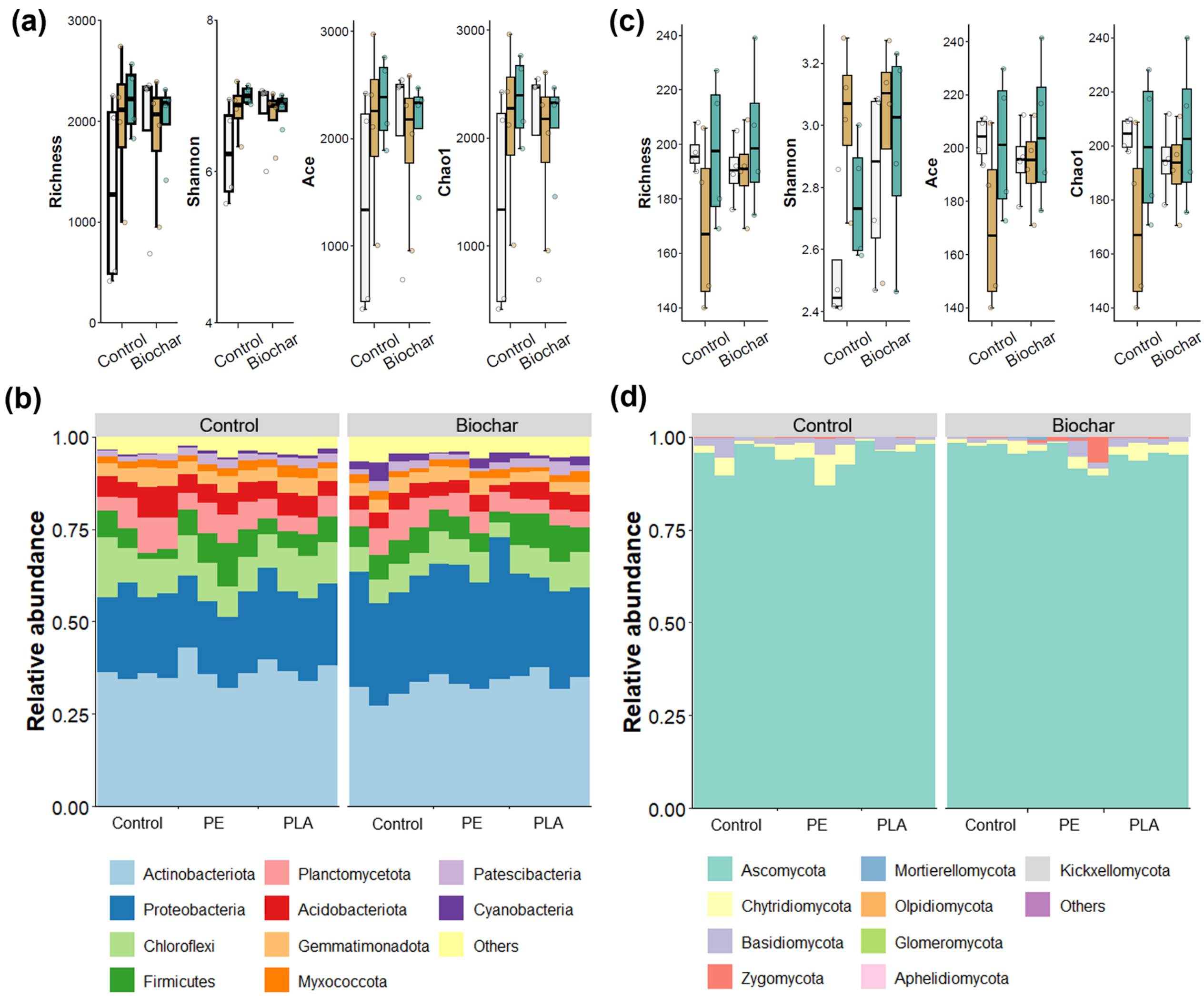


Fig. 3 Boxplots of α-diversity and stacked plots of relative abundance of dominant phyla for bacterial communities (**a**, **b**) and fungal communities (**c**, **d**). The phylum whose relative abundance is not in the top 10 is labeled "others". The line in the box represents the median of all data

biochar application positively influenced plant growth and N_2O emissions (path coefficients = 0.766 and 0.684) while negatively affecting N processes (including PNR, PDR, and AOA nitrification; path coefficient = −0.98). Soil physiochemical properties had a negative impact on N functional genes (path coefficient = −0.650) but were positively related to N processes (path coefficient = 0.945), both of which directly affected N_2O emissions (path coefficients = 0.296 and 0.596).

4 Discussion

4.1 Response of plant biomass to biochar and microplastics

The impact of MPs on plant biomass has been extensively examined, but a consensus remains elusive. In line with our study, some previous studies have found no significant effect (Greenfield et al. 2022; Judy et al. 2019; Meng et al. 2021). However, other studies have reported both positive (de Souza Machado et al. 2019; Hernandez-Arenas et al. 2021; Lozano et al. 2021a, 2021b) and negative impacts (Boots et al. 2019; Khan et al. 2024; Pignattelli et al. 2021; Qi et al. 2018). These discrepancies can be primarily ascribed to the heterogeneity of experimental conditions, such as soil properties, study durations, and the characteristics of the MPs used (Baho et al. 2021). Previous studies have demonstrated the importance of MP concentration in altering plant growth, primarily through changes in soil structure and hydrological properties (Boots et al. 2019; de Souza Machado et al. 2019; Lozano et al. 2021b; Ma et al. 2020). Specifically, at relatively low MP concentrations of large particles (millimeter-sized), PE and PLA (> 1 mm; 0.05% *w/w*) led to no direct toxicity to plants, resulting in a neglectable effect on plant biomass (Table 1). In contrast, micrometer-sized PP at high

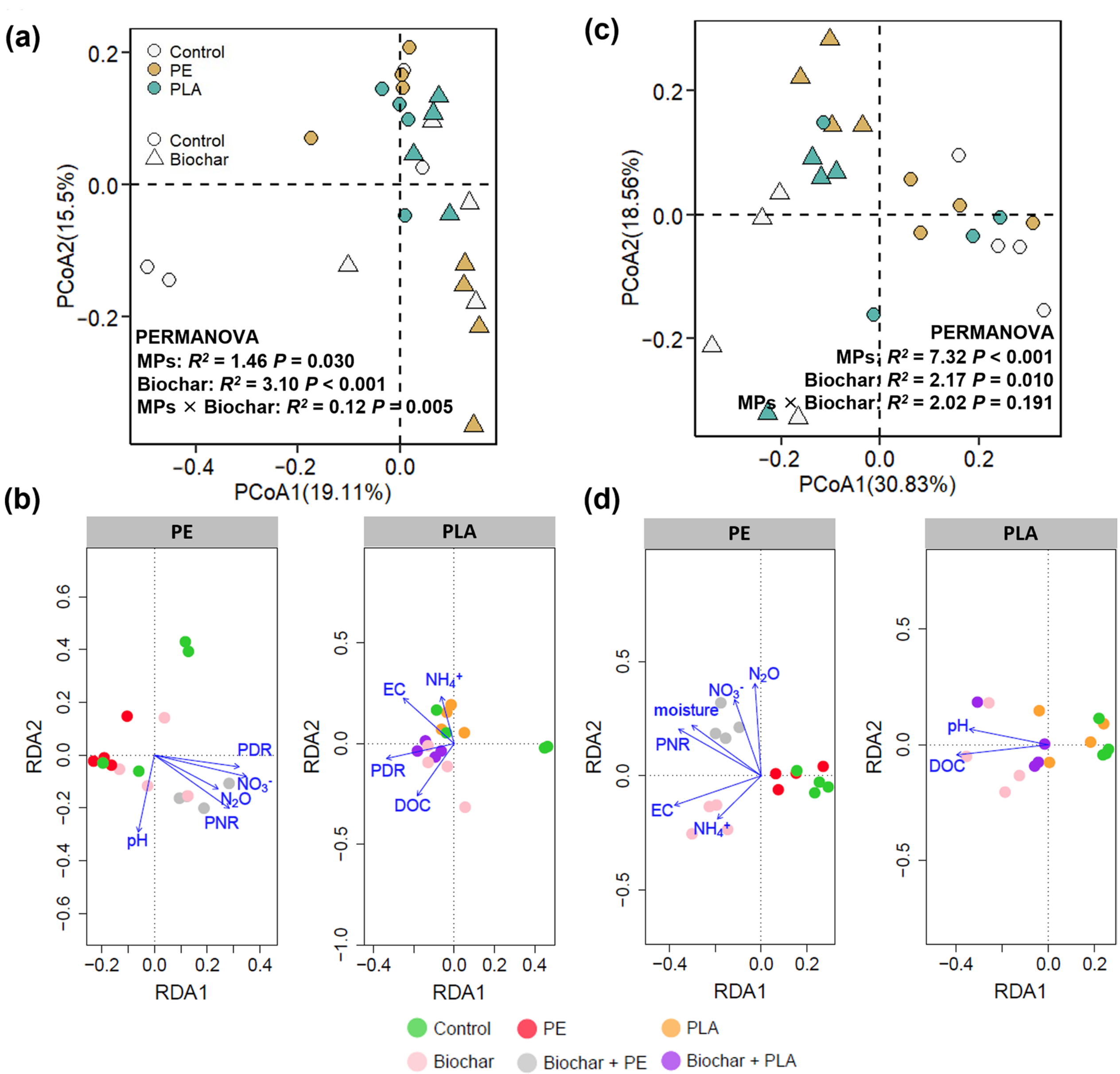


Fig. 4 Principal component analysis (PCoA) using the Bray–Curtis distances and corresponding redundancy analysis (RDA) of the correlations with soil properties grouped by MP type for bacterial (**a**, **b**) and fungal communities (**c**, **d**). PNR, potential nitrification rate; PDR, potential denitrification rate

concentrations (1 μm, 1% *w/w*) inhibited N uptake and vegetative growth by directly damaging root cells (Liu et al. 2023). The effects also varied considerably by plant species and soil health. For instance, the presence of PE, PP, and PS (< 10 μm, 0.1% *w/w*) was found to reduce lettuce biomass with decreased resistance to pathogens in the soil (Lian et al. 2024). Given root growth conditions serve as early indicators of plant health, previous studies of PE, PLA (0.5% *w/w*), and PLA-PBAT (≥ 1.5% *w/w*) showed adverse effects on root growth traits by interfering with soil microorganisms in the rhizosphere (Meng et al. 2023). Similar adverse effects were observed in our study with PLA treatments, suggesting the alteration of plant growth occurred to some extent. Furthermore, the MPs used in our study had a high C-to-hydrogen ratio, which is not conducive to plant growth (Xiao et al. 2016).

Besides, biochar addition significantly increased the aboveground biomass, independent of the microplastic application. This positive effect is consistent with

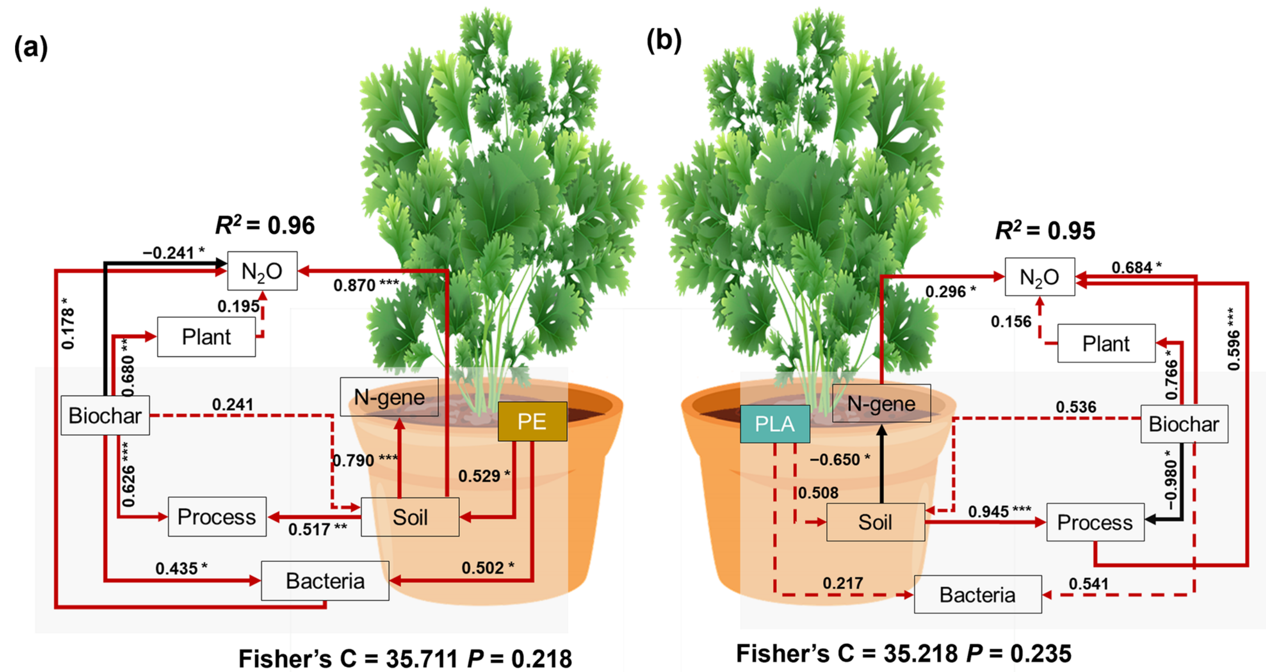


Fig. 5 Structural equation modeling depicting potential causal relationships between soil physical properties, microbial β-diversity, N genes, N processes, and N_2O emissions with the addition of PE (**a**) and PLA (**b**). The width of the arrow indicates the strength of the potential causal effect. The red and blue arrows indicate the positive and negative relationships between the indicators, respectively. The numbers above the arrows indicate the path coefficients. R^2 denotes the amount of variance explained by the model for the response variables of N_2O emissions. Significant ($P < 0.05$) and insignificant paths were distinguished by solid and dashed lines, respectively

the previous studies, although the extent of the effect can vary depending on soil conditions (Joseph et al. 2021). The mechanism underlying biochar-induced plant growth may involve N transformation, which benefits plant growth by increasing N availability of NH_4^+ in the soil (Table 1). To our knowledge, a few studies have shown an increase in plant biomass of polyvinyl chloride (PVC)-MPs and PP-MPs applied with biochar amendment (Khalid et al. 2023), while others have reported negative effects with PE and PP-MPs under biochar application (Miao et al. 2023). The effect of biochar and MPs on plant performance may depend on their concentrations and types and specific environmental conditions. For instance, following biochar application, soils with a higher DOC may render microorganisms inactive to new C inputs from MPs. This aligns with the evidence that non-additive interactions, either synergistic or antagonistic, are commonly found when biochar is applied with organic amendments (Bonanomi et al. 2017).

4.2 Interactive effects of biochar and microplastics on N_2O emissions

Soil N_2O is primarily produced as an intermediate during various N transformation processes (Firestone and Davidson 1989). In our study, we observed a decrease in N_2O emissions in the presence of biochar or MPs, which was closely associated with the decreased nitrification and denitrification processes in the soil. This was evidenced by reduced mineral N content, PNR, PDR, and the relative abundance of N functional genes. Especially for PLA, N functional genes of N mineralization, nitrification, and denitrification reduced regardless of biochar presence. Our findings are consistent with previous studies showing that biochar consistently reduces N_2O emissions in agricultural soils by decreasing denitrification (Cayuela et al. 2013). Besides, in our study, the decrease in N_2O emissions in biochar-added soil can be attributed to the increased DOC by higher C aromaticity, which is inert to microorganisms, limiting the abundance of N functional genes (Benbi and Brar 2021; Novair et al. 2023).

For MPs addition, our findings are consistent with those studies in agricultural systems with N fertilization, indicating a decrease in N_2O emissions, regardless of plant presence (Khan et al. 2024; Ren et al. 2020; Rillig et al. 2021), attributed to the reduced N availability. The input of microplastic-derived C causes the perturbation of soil microbial functions in the C and N cycles (Seeley et al. 2020). Although plastics were considered recalcitrant, we observed surface erosion in both biodegradable

(PLA) and petroleum-based (PE) plastics in SEM images, as verified by FTIR analysis (Fig. S2). Therefore, MPs can interfere with the soil C pool by the increased microbial communities of hydrocarbon-degrading species, distorting soil organic matter turnover to a certain degree, as the mineralization ratio of microplastic C is close to soil organic matter and can exceed that of biochar C (Yang et al. 2022). In N-limited soil of farmlands, N fertilization accelerates microbial mineralization of organic C, releasing CO_2 and thereby reducing the C sequestration potential and DOC of the soil (Sheng et al. 2016). As DOC generally correlates positively with microbial activities and N mineralization, N turnover processes were suppressed to minimize N_2O emissions (Campbell et al. 2021). However, the extent to which specific microorganisms utilize MP-C and its associated impact on soil N cycling depends on the polymer and environmental conditions (Shen et al. 2023). Contrary to our results, some studies have indicated that MPs have no significant or positive effect on N_2O emissions, primarily due to an increased denitrification rate (Su et al. 2023). PE over 0.5% (*w/w*) increased denitrification of N_2O production from paddy soils by improving soil aeration and the *nir*S gene (Yu et al. 2022, 2023). An increase in denitrification rates and *nir*S abundance was also found in PLA treatments, supported by enhanced NO_3^- as substrates in salt sediment microcosm incubation with decreasing N_2O emissions (Seeley et al. 2020). Besides soil water content, other experimental conditions, including the type of MPs used, can also explain these inconsistent responses in the N cycle. Compared with PLA, PE showed greater suppression of N_2O emissions in agricultural systems, consistent with a three-year field study where PE reduced more N_2O emissions than PHBV (poly(3-hydroxybutyrate-co-3-hydroxyvalerate), a biodegradable polymer (Greenfield et al. 2022). This was supported, firstly, by higher plant biomass observed in soil treated with PE, where most of N was taken up from the soil to plants, reducing N loss from the soil (Cameron et al. 2013). Secondly, in PE treatments, we observed that despite the increase in the abundance and proportion of AOB-driven autotrophic nitrification, which enhanced N_2O emissions, there was also a higher abundance of *nos*Z, leading to more conversion of N_2O into N_2, thus resulting in a net decrease compared to the PLA treatments (Butterbach-Bahl et al. 2013). Meantime, the increased abundance of *nosZ* was aligned with the increased *Firmicutes*, which contained bacillus species, significantly promoting complete denitrification of the N_2O-reducing bacteria (Wu et al. 2018). Thus, the extent to which MPs affect which types of N-related microbes depends on the type of MP used. MPs can provide extracellular polymeric substances (EPS) of biopolymer excretion and moderate the microbial habitation of soil aeration and moisture, resulting in differences in microbial performance during N turnover, thus leading to variations in N_2O emissions (Chen et al. 2022; Wang et al. 2024). Previous studies on MPs effects on EPS were primarily focused on aquatic systems, which were proved to drive highly complex and dynamic microbial assemblage successions with nutrient cycling through microbe-mediated processes, thus impacting the N behavior (Crouzet et al. 2019). However, the study on EPS of MPs in soils is still scarce and needs further investigations.

Under biochar application, the mitigation of N_2O emissions caused by MPs was lessened and even reversed, leading to an increase with PE addition. This outcome was supported by the rise in soil NO_3^-, which serves as a substrate for denitrification, thereby limiting the decrease in PDR induced by biochar. In our research of peanutshell biochar, PE addition increased the abundance of *nifH* and *nirK* ($P < 0.1$), which enhanced N_2O production, while PLA addition reduced *nosZ* abundance (though not significant), which decreased N_2O by 6.8%. Interestingly, previous studies demonstrated that N_2O emissions were increased with PE and PET application (petroleum-based MPs) under straw biochar application by reducing *nos*Z abundance (Han et al. 2022; Li et al. 2022a) or growing *nir*S and decreasing *nif*H (Chen et al. 2024). Thus, besides MP types, the change in N_2O emissions varied by biochar type, which has also been proved since PE in straw biochar reduced the abundance of *nos*Z while increasing in manure biochar (Li et al. 2022b). Since research on the combined effects of biochar and MPs on N_2O emissions is limited, varying by different polymer types, biochar types, and soil environments, further investigation is warranted. Based on SEM modeling, the combined application of PE and biochar significantly regulated the N_2O emissions when co-applied, while PLA showed no significant direct impact on N_2O emissions under biochar application. When PE and biochar were co-applied, bacterial pathways emerged as a key factor positively related to N_2O emissions. Biochar directly inhibited N_2O emissions but was positively associated with N_2O emissions through changes in bacterial communities. Meanwhile, PE significantly increased N_2O emissions by positively correlating with bacterial communities and soil physical properties. Like paddy soil, adding biochar (5% *w/w*) and/or PE addition (0.5–1% *w/w*) interferes with N_2O emissions by altering soil chemical properties, microbial community structure, and functional gene abundance (Zhang et al. 2023). When biochar coexisted with PLA, the reduction of N_2O emissions was directly related to biochar and indirectly influenced by soil N process (AOA-driven nitrification) and N functional genes, which potentially have a negative relationship with soil physical properties and the presence of biochar. The direct effect of the PLA application was not significant. Overall, unlike PE, when biochar and MPs coexist, the impact of PLA in the soil would be masked by biochar application. Considering the economic remediation pathways of the soil itself, the impact of MPs on soil N biogeochemical processes and the related microbial pathways in terrestrial ecosystems should be considered as the focus of future research on biochar remediation of microplastic pollution in agriculture systems.

4.3 Effects of biochar and microplastics on soil microbial communities

Given that MPs and biochar have not been widely found in farmlands, concerns have emerged regarding their potential impact on soil microorganisms (Rillig et al. 2019). While changes in microbial mechanisms have been observed in the soil N cycle, our study found no significant effect on the α-diversity of microbial communities with the addition of MPs and biochar. This finding is consistent with incubation studies (0.03 to 2 mm PE and < 2 mm polyester; 1% *w/w* and 50 mg L^{-1}) (Guo et al. 2021; Huang et al. 2019; Li et al. 2021; Ng et al. 2021) and pot experiments (< 2 mm PE, PET, and PVC; 1% *w/w*) (Judy et al. 2019). However, at high doses of MP application in previous studies, the microbial α-diversity was reduced (< 2 mm PE, PET, PVC, and polyester; > 1% *w/w*) (Fei et al. 2020; Guo et al. 2021; Judy et al. 2019; Ren et al. 2020). This dose-dependent effect may be attributed to the species-selectivity theory of microbial colonization in the plastisphere (Gaylarde et al. 2023), resulting in a simplified microbial composition and further driving diverse ecological processes of bulk soil (Ran et al. 2024; Zhou et al. 2021). We believe the MP dose, which indicates the proportion of plastispheres, relatives to the bulk soil, may be pivotal in determining microbial response. Moreover, ongoing research delves into the effects of various microplastic characteristics on soil microorganisms, including plastic type, shape, concentration, particle size, etc., yet the definitive effective index behind these characterizations remains undetermined.

Regarding microbial composition, biochar application increased the presence of *Ascomycota* in the soil samples, which is consistent with the previous studies (Zhu et al. 2019). *Ascomycota* can decompose and mineralize the aromatic C skeleton of biochar (De la Rosa et al. 2018; Zimmerman et al. 2011). Similar to the effect of biochar addition in other studies, the growth of *Zygomycota* was observed by PE application in biochar amendment soil, devoted to providing a better habitat for the survival of the specific phylum on recalcitrant C. However, the potential mechanism of fungal colonization on recalcitrant C and its effect on community composition remains unclear and needs more research. Adding biochar and MPs also increased *Firmicutes*, a phylum known for its adaptability to extreme conditions (Hayward et al. 2021). This increase can be attributed to the fact that many bacterial species of *Firmicutes* are capable of degrading plastics (Han et al. 2022), as observed previously (Yang et al. 2023; Lv et al. 2024). On the other hand, the presence of *Planctomycetes* decreased in the PLA treatment, which aligns with the reduction in *amo*A and *amo*B, as *Planctomycetes* play a role in anaerobic ammonia oxidation (Klotz et al. 2008; Stein 2011). Overall, the composition of the microbial community is shifted by biochar and MP application, implying the disturbance in the soil C pool.

The PCoA revealed that the composition of soil microbiomes, as indicated by β-diversity, was influenced by applying biochar and MPs and their combined effects. First, we observed fungal composition was more influenced than that of a bacterial community under biochar addition, which is consistent with a four-year biochar amendment (20 and 40 t ha^{-1}) study of rice paddy (Zheng et al. 2016) and supported by the apparent increase in the relative abundance of *Ascomycota* under biochar application, as mentioned above. Second, when biochar was not applied, PE significantly influenced the bacterial and fungal communities in the soil. However, in biochar-applied soil, PLA had a significant effect. This suggests that different types of MPs may exert varying selective pressures on soil microbial communities, meantime with the extent of the change depending on soil properties shifted by biochar amendment (Wang et al. 2023). The Monte Carlo permutation test of db-RDA emphasized that the nitrification and denitrification processes were the main factors determining the composition of bacterial communities in PLA treatments, while PE affected bacteria mainly by altering soil properties such as EC and DOC. Therefore, the different polymer components of the plastics result in various behaviors of microbial communities. Supporting this observation, both biochar and PE are recalcitrant to microorganisms, and the bacterial community compositions were altered by the induced change in soil N transformations (NO_3^-, PDR, PNR, and N_2O emissions) under the limited decomposition. However, C of PLA is microbially available, potentially resulting in hydrolysis reactions in soil, thereby altering soil EC and DOC, which became the main driving forces to bacterial composition shifts instead. Thus, more investigation on the driving mechanism of MPs on bacterial composition is needed, especially for different MP types, as the component change might altered differently to soil properties.

Given that fungal communities were more sensitive to biochar addition, the RDA of PLA treatments revealed that changes in pH and DOC were important in altering fungal community composition. The result is consistent with the persistent effect of single applied biochar in field experiments, indicating increased microbial C use efficiency and enhanced soil organic matter stabilization (Zheng et al. 2016). Therefore, we supposed that biochar takes advantage of PLA application on fungal communities.

5 Conclusions

In summary, our study presented insights into the differential effects of biochar, MPs, and their interactions on various key parameters of vegetable growth and soil health. Biochar increased the aboveground biomass of coriander by enhancing soil NH_4^+ availability, independent of the presence of MPs. Both biochar and MPs significantly reduced N_2O emissions by limiting N transformation processes through reductions in mineral N content and changes in related functional genes. While MP addition lowered DOC, inhibiting overall N mineralization and N turnover processes, the increase in DOC from biochar, characterized by higher C aromaticity, remained largely inert to microorganisms, leading to a reduction in N-related functional genes and N_2O emissions. The effect of biochar on N_2O emissions in the presence of MPs varied depending on the type of MP. PE exhibited higher N conversion of the increased *nifH* and *nirK* and bacterial diversity, whereas PLA decreased the *nosZ* gene when adding biochar. Although microbial α-diversity was not affected by biochar and MPs, β-diversity was significantly affected by the type of porous material and biochar presence. Introducing MP-derived C promoted hydrocarbon-degrading microbial communities, disrupting soil microbial functions in the C and N cycles. The separation of bacterial communities from PE was more pronounced with biochar application, which promoted the growth of inert C-preferring bacteria and provided additional habitat. In addition, significant correlations between MPs, biochar, soil parameters, and microbial community composition highlighted the intricate interactions among these factors. These findings deepen the understanding of how biochar and MPs interactively shape soil N cycling and microbial communities in plant–soil systems, contributing to optimizing sustainable environmental management practices in agriculture.

Supplementary Information

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Supplementary Material 1.

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Author contributions
ZH: Conceptualization, formal analysis, methodology, experimental operation, visualization, writing-original draft. QY: Experimental operation; RC: Experimental operation, writing—review and editing; JW: Experimental operation, methodology, supervision, funding acquisition, writing—reviewing and editing; XL: Conceptualization, supervision, writing—reviewing and editing. All author read and approved the final manuscript.

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Availability of data and materials
The datasets used or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Competing interests
The authors have no relevant financial or non-financial interests to disclose.

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References

Baho DL, Bundschuh M, Futter MN (2021) Microplastics in terrestrial ecosystems: moving beyond the state of the art to minimize the risk of ecological surprise. Glob Change Biol 27:3969–3986. https://doi.org/10.1111/gcb.15724

Benbi DK, Brar K (2021) Pyrogenic conversion of rice straw and wood to biochar increases aromaticity and carbon accumulation in soil. Carbon Management 12:385–397. https://doi.org/10.1080/17583004.2021.1962409

Bonanomi G, Ippolito F, Cesarano G, Nanni B, Lombardi N, Rita A, Saracino A, Scala F (2017) Biochar as plant growth promoter: better off alone or mixed with organic amendments? Front Plant Sci 8:1570. https://doi.org/10.3389/fpls.2017.01570

Boots B, Russell CW, Green DS (2019) Effects of microplastics in soil ecosystems: above and below ground. Environ Sci Technol 53:11496–11506. https://doi.org/10.1021/acs.est.9b03304

Butterbach-Bahl K, Baggs EM, Dannenmann M, Kiese R, Zechmeister-Boltenstern S (2013) Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philos Trans R Soc B Biol Sci 368:20130122

Cameron KC, Di HJ, Moir JL (2013) Nitrogen losses from the soil/plant system: a review. Ann Appl Biol 162:145–173. https://doi.org/10.1111/aab.12014

Campbell TP, Ulrich DEM, Toyoda J, Thompson J, Munsky B, Albright MBN, Bailey VL, Tfaily MM, Dunbar J (2021) Microbial communities influence soil dissolved organic carbon concentration by altering metabolite composition. Front Microbiol 12:799014. https://doi.org/10.3389/fmicb.2021.799014

Castellini M, Giglio L, Niedda M, Palumbo AD, Ventrella D (2015) Impact of biochar addition on the physical and hydraulic properties of a clay soil. Soil Tillage Res 154:1–13. https://doi.org/10.1016/j.still.2015.06.016

Cayuela ML, Sanchez-Monedero MA, Roig A, Hanley K, Enders A, Lehmann J (2013) Biochar and denitrification in soils: when, how much and why does biochar reduce N(2)O emissions? Sci Rep 3:1732. https://doi.org/10.1038/srep01732

Chai B, Yin H, Xiao T, Xiao E, Dang Z, Pan K (2024) Effects of microplastics on endophytes in different niches of Chinese flowering cabbage (*Brassica*

campestris). J Agric Food Chem 72:4679–4688. https://doi.org/10.1021/acs.jafc.3c09092
Chen C, Pan J, Xiao S, Wang J, Gong X, Yin G, Hou L, Liu M, Zheng Y (2022) Microplastics alter nitrous oxide production and pathways through affecting microbiome in estuarine sediments. Water Res 221:118733. https://doi.org/10.1016/j.watres.2022.118733
Chen Y, Li Y, Liang X, Lu S, Ren J, Zhang Y, Han Z, Gao B, Sun K (2024) Effects of microplastics on soil carbon pool and terrestrial plant performance. Carbon Res. https://doi.org/10.1007/s44246-024-00124-1
Clayton H, Arah JRM, Smith KA (1994) Measurement of nitrous oxide emission from fertilized grassland using micrometeorological techniques. J Geophys Res Atmos 99:16599–16607
Crouzet O, Consentino L, Pétraud J-P, Marrauld C, Aguer J-P, Bureau S, Le Bourvellec C, Touloumet L, Bérard A (2019) Soil photosynthetic microbial communities mediate aggregate stability: influence of cropping systems and herbicide use in an agricultural soil. Front Microbiol. https://doi.org/10.3389/fmicb.2019.01319
de Klein C, Harvey M (2012) Nitrous oxide chamber methodology guidelines. Ministry for Primary Industries
de Souza Machado AA, Lau CW, Kloas W, Bergmann J, Bachelier JB, Faltin E, Becker R, Gorlich AS, Rillig MC (2019) Microplastics can change soil properties and affect plant performance. Environ Sci Technol 53:6044–6052. https://doi.org/10.1021/acs.est.9b01339
De la Rosa JM, Miller AZ, Knicker H (2018) Soil-borne fungi challenge the concept of long-term biochemical recalcitrance of pyrochar. Sci Rep 8:2896. https://doi.org/10.1038/s41598-018-21257-5
Fan P, Yu H, Xi B, Tan W (2022) A review on the occurrence and influence of biodegradable microplastics in soil ecosystems: are biodegradable plastics substitute or threat? Environ Int 163:107244. https://doi.org/10.1016/j.envint.2022.107244
Fei Y, Huang S, Zhang H, Tong Y, Wen D, Xia X, Wang H, Luo Y, Barcelo D (2020) Response of soil enzyme activities and bacterial communities to the accumulation of microplastics in an acid cropped soil. Sci Total Environ 707:135634. https://doi.org/10.1016/j.scitotenv.2019.135634
Feng Y, Han L, Sun H, Zhu D, Xue L, Jiang ZT, Poinern GEJ, Lu Q, Feng Y, Xing B (2022b) Every coin has two sides: Continuous and substantial reduction of ammonia volatilization under the coexistence of microplastics and biochar in an annual observation of rice-wheat rotation system. Sci Total Environ 847:157635. https://doi.org/10.1016/j.scitotenv.2022.157635
Firestone MK, Davidson EA (1989) Microbiological basis of NO and N_2O production and consumption in soil. Exchange Trace Gases between Terrestrial Ecosyst Atmos 47:7–21
Gao B, Yao H, Li Y, Zhu Y (2021) Microplastic addition alters the microbial community structure and stimulates soil carbon dioxide emissions in vegetable-growing soil. Environ Toxicol Chem 40:352–365. https://doi.org/10.1002/etc.4916
Gaylarde CC, de Almeida MP, Neves CV, Neto JAB, da Fonseca EM (2023) The importance of biofilms on microplastic particles in their sinking behavior and the transfer of invasive organisms between ecosystems. Micro 3:320–337. https://doi.org/10.3390/micro3010022
Ge J, Li H, Liu P, Zhang Z, Ouyang Z, Guo X (2021) Review of the toxic effect of microplastics on terrestrial and aquatic plants. Sci Total Environ 791:148333. https://doi.org/10.1016/j.scitotenv.2021.148333
Greenfield LM, Graf M, Rengaraj S, Bargiela R, Williams G, Golyshin PN, Chadwick DR, Jones DL (2022) Field response of N_2O emissions, microbial communities, soil biochemical processes and winter barley growth to the addition of conventional and biodegradable microplastics. Agricult Ecosyst Environ. https://doi.org/10.1016/j.agee.2022.108023
Guo QQ, Xiao MR, Ma Y, Niu H, Zhang GS (2021) Polyester microfiber and natural organic matter impact microbial communities, carbon-degraded enzymes, and carbon accumulation in a clayey soil. J Hazard Mater 405:124701. https://doi.org/10.1016/j.jhazmat.2020.124701
Han L, Chen L, Li D, Ji Y, Feng Y, Feng Y, Yang Z (2022) Influence of polyethylene terephthalate microplastic and biochar co-existence on paddy soil bacterial community structure and greenhouse gas emission. Environ Pollut 292:118386. https://doi.org/10.1016/j.envpol.2021.118386
Han Y, Teng Y, Wang X, Wen D, Gao P, Yan D, Yang N (2024) Biodegradable PBAT microplastics adversely affect pakchoi (*Brassica chinensis* L.) growth and the rhizosphere ecology: focusing on rhizosphere microbial community composition, element metabolic potential, and root exudates. Sci Total Environ 912:169048. https://doi.org/10.1016/j.scitotenv.2023.169048
Hart SC, Stark JM, Davidson EA, Firestone MK, Weaver RW, Peter SA, Bezdicek BD, Ali SS, Wollum TA (1994) Methods of Soil Analysis Part 2 Microbiological and Biochemical Properties. In: Nitrogen mineralization immobilization and nitrification soil science society of America. Madison WI, USA, pp 985-1018
Hayward MK, Dewey ED, Shaffer KN, Huntington AM, Burchell BM, Stokes LM, Alexander BC, George JE, Kempher ML, Joye SB, Madigan MT (2021) Cultivation and characterization of snowbound microorganisms from the South Pole. Extremophiles 25:159-172. https://doi.org/10.1007/s00792-021-01218-z
Hernandez-Arenas R, Beltran-Sanahuja A, Navarro-Quirant P, Sanz-Lazaro C (2021) A review ofmicroplastics pollution in the soil and terrestrial ecosystems: a global and Bangladesh perspective. Environ Pollut 268:115779. https://doi.org/10.1016/j.envpol.2020.115779
Hu X, Gu H, Sun X, Wang Y, Liu J, Yu Z, Li Y, Jin J, Wang G (2023) Distinct influence of conventional and biodegradable microplastics on microbe-driving nitrogen cycling processes in soils and plastispheres as evaluated by metagenomic analysis. J Hazard Mater. https://doi.org/10.1016/j.jhazmat.2023.131097
Huang Y, Zhao Y, Wang J, Zhang M, Jia W, Qin X (2019) LDPE microplastic films alter microbial community composition and enzymatic activities in soil. Environ Pollut 254:112983. https://doi.org/10.1016/j.envpol.2019.112983
Joseph S, Cowie AL, Van Zwieten L, Bolan N, Budai A, Buss W, Cayuela ML, Graber ER, Ippolito JA, Kuzyakov Y et al (2021) How biochar works, and when it doesn't: a review of mechanisms controlling soil and plant responses to biochar. Glob Change Biol Bioenergy 13:1731–1764. https://doi.org/10.1111/gcbb.12885
Judy JD, Williams M, Gregg A, Oliver D, Kumar A, Kookana R, Kirby JK (2019) Microplastics in municipal mixed-waste organic outputs induce minimal short to long-term toxicity in key terrestrial biota. Environ Pollut 252:522–531. https://doi.org/10.1016/j.envpol.2019.05.027
Khalid N, Aqeel M, Noman A (2020) Microplastics could be a threat to plants in terrestrial systems directly or indirectly. Environ Pollut 267:115653. https://doi.org/10.1016/j.envpol.2020.115653
Khalid AR, Shah T, Asad M, Ali A, Samee E, Adnan F, Bhatti MF, Marhan S, Kammann CI, Haider G (2023) Biochar alleviated the toxic effects of PVC microplastic in a soil-plant system by upregulating soil enzyme activities and microbial abundance. Environ Pollut 332:121810. https://doi.org/10.1016/j.envpol.2023.121810
Khan KY, Tang Y, Cheng P, Song Y, Li X, Lou J, Iqbal B, Zhao X, Hameed R, Li G, Du D (2024) Effects of degradable and non-degradable microplastics and oxytetracycline co-exposure on soil N_2O and CO_2 emissions. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2024.105331
Klotz MG, Schmid MC, Strous M, Op Den Camp HJ, Jetten MS, Hooper AB (2008) Evolution of an octahaem cytochrome c protein family that is key to aerobic and anaerobic ammonia oxidation by bacteria. Environ Microbiol 10(11):3150-3163. https://doi.org/10.1111/j.1462-2920.2008.01733.x
Lefcheck JS, Freckleton R (2015) piecewiseSEM: piecewise structural equation modelling in r for ecology, evolution, and systematics. Methods Ecol Evol 7:573–579. https://doi.org/10.1111/2041-210x.12512
Li HZ, Zhu D, Lindhardt JH, Lin SM, Ke X, Cui L (2021) Long-term fertilization history alters effects of microplastics on soil properties, microbial communities, and functions in diverse farmland ecosystem. Environ Sci Technol 55:4658–4668. https://doi.org/10.1021/acs.est.0c04849
Li X, Yao S, Wang Z, Jiang X, Song Y, Chang SX (2022a) Microplastic and biochar coexistence decreases their stimulation on global warming potential resulting from soil greenhouse gas emissions. SSRN Electr J. https://doi.org/10.2139/ssrn.4056815
Li X, Yao S, Wang Z, Jiang X, Song Y, Chang SX (2022b) Polyethylene microplastic and biochar interactively affect the global warming potential of soil greenhouse gas emissions. Environ Pollut 315:120433. https://doi.org/10.1016/j.envpol.2022.120433
Lian Y, Shi R, Liu J, Zeb A, Wang Q, Wang J, Yu M, Li J, Zheng Z, Ali N et al (2024) Effects of polystyrene, polyethylene, and polypropylene microplastics on the soil-rhizosphere-plant system: phytotoxicity, enzyme activity, and microbial community. J Hazard Mater 465:133417. https://doi.org/10.1016/j.jhazmat.2023.133417
Liu Y, Xu F, Ding L, Zhang G, Bai B, Han Y, Xiao L, Song Y, Li Y, Wan S, Li G (2023) Microplastics reduce nitrogen uptake in peanut plants by damaging root cells and impairing soil nitrogen cycling. J Hazard Mater 443:130384. https://doi.org/10.1016/j.jhazmat.2022.130384

Lozano YM, Aguilar-Trigueros CA, Onandia G, Maaß S, Zhao T, Rillig MC, Macinnis-Ng C (2021a) Effects of microplastics and drought on soil ecosystem functions and multifunctionality. J Appl Ecol 58:988–996. https://doi.org/10.1111/1365-2664.13839
Lozano YM, Lehnert T, Linck LT, Lehmann A, Rillig MC (2021b) Microplastic shape, polymer type, and concentration affect soil properties and plant biomass. Front Plant Sci 12:616645. https://doi.org/10.3389/fpls.2021.616645
Lv S, Li Y, Zhao S, Shao Z (2024) Biodegradation of typical plastics: from microbial diversity to metabolic mechanisms. Int J Mol Sci 25(1):593. https://doi.org/10.3390/ijms25010593
Ma J, Sheng GD, O'Connor P (2020) Microplastics combined with tetracycline in soils facilitate the formation of antibiotic resistance in the *Enchytraeus crypticus* microbiome. Environ Pollut 264:114689. https://doi.org/10.1016/j.envpol.2020.114689
Meng F, Yang X, Riksen M, Xu M, Geissen V (2021) Response of common bean (*Phaseolus vulgaris* L.) growth to soil contaminated with microplastics. Sci Total Environ 755:142516. https://doi.org/10.1016/j.scitotenv.2020.142516
Meng F, Harkes P, van Steenbrugge JJM, Geissen V (2023) Effects of microplastics on common bean rhizosphere bacterial communities. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2022.104649
Miao J, Chen Y, Zhang E, Yang Y, Sun K, Gao B (2023) Effects of microplastics and biochar on soil cadmium availability and wheat plant performance. GCB Bioenergy 15:1046–1057. https://doi.org/10.1111/gcbb.13083
Moshood TD, Nawanir G, Mahmud F, Mohamad F, Ahmad MH, AbdulGhani A (2022) Sustainability of biodegradable plastics: new problem or solution to solve the global plastic pollution? Curr Res Green Sustain Chem. https://doi.org/10.1016/j.crgsc.2022.100273
Ng EL, Lin SY, Dungan AM, Colwell JM, Ede S, Huerta Lwanga E, Meng K, Geissen V, Blackall LL, Chen D (2021) Microplastic pollution alters forest soil microbiome. J Hazard Mater 409:124606. https://doi.org/10.1016/j.jhazmat.2020.124606
Norton JM, Stark JM (2011) Regulation and measurement of nitrification in terrestrial systems. Methods Enzymol 486:343–368
Novair SB, Cheraghi M, Faramarzi F, Lajayer BA, Senapathi V, Astatkie T, Price G (2023) Reviewing the role of biochar in paddy soils: an agricultural and environmental perspective. Ecotoxicol Environ Saf 263:115228
OECD (2022) Global plastics outlook: policy scenarios to 2060 (OECD). OECD. https://doi.org/10.1787/aa1edf33-en
Palansooriya KN, Sang MK, El-Naggar A, Shi L, Chang SX, Sung J, Zhang W, Ok YS (2023) Low-density polyethylene microplastics alter chemical properties and microbial communities in agricultural soil. Sci Rep 13:16276. https://doi.org/10.1038/s41598-023-42285-w
Pignattelli S, Broccoli A, Piccardo M, Terlizzi A, Renzi M (2021) Effects of polyethylene terephthalate (PET) microplastics and acid rain on physiology and growth of *Lepidium sativum*. Environ Pollut 282:116997. https://doi.org/10.1016/j.envpol.2021.116997
Qi Y, Yang X, Pelaez AM, Huerta Lwanga E, Beriot N, Gertsen H, Garbeva P, Geissen V (2018) Macro- and micro- plastics in soil-plant system: effects of plastic mulch film residues on wheat (*Triticum aestivum*) growth. Sci Total Environ 645:1048–1056. https://doi.org/10.1016/j.scitotenv.2018.07.229
Ran T, Liao H, Zhao Y, Li J (2024) Soil plastisphere interferes with soil bacterial community and their functions in the rhizosphere of pepper (*Capsicum annuum* L.). Ecotoxicol Environ Saf 270:115946. https://doi.org/10.1016/j.ecoenv.2024.115946
Ren X, Tang J, Liu X, Liu Q (2020) Effects of microplastics on greenhouse gas emissions and the microbial community in fertilized soil. Environ Pollut 256:113347. https://doi.org/10.1016/j.envpol.2019.113347
Rillig MC, de Souza Machado AA, Lehmann A, Klumper U (2019) Evolutionary implications of microplastics for soil biota. Environ Chem 16:3–7. https://doi.org/10.1071/EN18118
Rillig MC, Hoffmann M, Lehmann A, Liang Y, Lück M, Augustin J (2021) Microplastic fibers affect dynamics and intensity of CO2 and N2O fluxes from soil differently. Microplast Nanoplast. https://doi.org/10.1186/s43591-021-00004-0
Seeley ME, Song B, Passie R, Hale RC (2020) Microplastics affect sedimentary microbial communities and nitrogen cycling. Nat Commun 11:2372. https://doi.org/10.1038/s41467-020-16235-3
Serrano-Ruiz H, Martin-Closas L, Pelacho AM (2021) Biodegradable plastic mulches: impact on the agricultural biotic environment. Sci Total Environ 750:141228. https://doi.org/10.1016/j.scitotenv.2020.141228
Shen H, Sun Y, Duan H, Ye J, Zhou A, Meng H, Zhu F, He H, Gu C (2023) Effect of PVC microplastics on soil microbial community and nitrogen availability under laboratory-controlled and field-relevant temperatures. Appl Soil Ecol. https://doi.org/10.1016/j.apsoil.2022.104794
Sheng Y, Zhu L (2018) Biochar alters microbial community and carbon sequestration potential across different soil pH. Sci Total Environ 622–623:1391–1399. https://doi.org/10.1016/j.scitotenv.2017.11.337
Sheng Y, Zhan Y, Zhu L (2016) Reduced carbon sequestration potential of biochar in acidic soil. Sci Total Environ 572:129–137. https://doi.org/10.1016/j.scitotenv.2016.07.140
Stein LY (2011) Surveying N_2O-producing pathways in bacteria. Methods Enzymol 486:131–152
Su P, Gao C, Zhang X, Zhang D, Liu X, Xiang T, Luo Y, Chu K, Zhang G, Bu N, Li Z (2023) Microplastics stimulated nitrous oxide emissions primarily through denitrification: a meta-analysis. J Hazard Mater 445:130500. https://doi.org/10.1016/j.jhazmat.2022.130500
Tanure MMC, da Costa LM, Huiz HA, Fernandes RBA, Cecon PR, Pereira Junior JD, da Luz JMR (2019) Soil water retention, physiological characteristics, and growth of maize plants in response to biochar application to soil. Soil Tillage Res 192:164–173. https://doi.org/10.1016/j.still.2019.05.007
Trivedi AK, Gupta MK, Singh H (2023) PLA based biocomposites for sustainable products: a review. Adv Ind Eng Polym Res. https://doi.org/10.1016/j.aiepr.2023.02.002
UNEP (2022) Historic day in the campaign to beat plastic pollution: nations commit to develop a legally binding agreement. https://www.unep.org/news-and-stories/press-release/historic-day-campaign-beat-plastic-pollution-nations-commit-develop.
Wang W, Ge J, Yu X, Li H (2020) Environmental fate and impacts of microplastics in soil ecosystems: progress and perspective. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2019.134841
Wang F, Wang Q, Adams CA, Sun Y, Zhang S (2022a) Effects of microplastics on soil properties: current knowledge and future perspectives. J Hazard Mater 424:127531. https://doi.org/10.1016/j.jhazmat.2021.127531
Wang Q, Feng X, Liu Y, Li W, Cui W, Sun Y, Zhang S, Wang F, Xing B (2023) Response of peanut plant and soil N-fixing bacterial communities to conventional and biodegradable microplastics. J Hazard Mater 459:132142. https://doi.org/10.1016/j.jhazmat.2023.132142
Wang W, Zhang Z, Gao J, Wu H (2024) The impacts of microplastics on the cycling of carbon and nitrogen in terrestrial soil ecosystems: progress and prospects. Sci Total Environ 915:169977. https://doi.org/10.1016/j.scitotenv.2024.169977
Wu S, Zhuang G, Bai Z, Cen Y, Xu S, Sun H, Han X, Zhuang X (2018) Mitigation of nitrous oxide emissions from acidic soils by *Bacillus amyloliquefaciens*, a plant growth-promoting bacterium. Glob Change Biol 24:2352–2365
Xiao X, Chen Z, Chen B (2016) H/C atomic ratio as a smart linkage between pyrolytic temperatures, aromatic clusters and sorption properties of biochars derived from diverse precursory materials. Sci Rep 6:22644
Yang F, Wang C, Sun H (2021) A comprehensive review of biochar-derived dissolved matters in biochar application: production, characteristics, and potential environmental effects and mechanisms. J Environ Chem Eng 9:105258
Yang Y, Sun K, Han L, Chen Y, Liu J, Xing B (2022) Biochar stability and impact on soil organic carbon mineralization depend on biochar processing, aging and soil clay content. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2022.108657
Yang X, Wen P, Yang Y, Jia P, Li W, Pei D (2023) Plastic biodegradation by in vitro environmental microorganisms and in vivo gut microorganisms of insects. Front Microbiol 13:1001750
Yu Y, Li X, Feng Z, Xiao M, Ge T, Li Y, Yao H (2022) Polyethylene microplastics alter the microbial functional gene abundances and increase nitrous oxide emissions from paddy soils. J Hazard Mater 432:128721. https://doi.org/10.1016/j.jhazmat.2022.128721
Yu Y, Li X, Fan H, Li Y, Yao H (2023) Dose effect of polyethylene microplastics on nitrous oxide emissions from paddy soils cultivated for different periods. J Hazard Mater 453:131445. https://doi.org/10.1016/j.jhazmat.2023.131445
Zhang S, Pei L, Zhao Y, Shan J, Zheng X, Xu G, Sun Y, Wang F (2023) Effects of microplastics and nitrogen deposition on soil multifunctionality, particularly C and N cycling. J Hazard Mater 451:131152
Zheng J, Chen J, Pan G, Liu X, Zhang X, Li L, Bian R, Cheng K, Jinwei Z (2016) Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice

paddy from southwest China. Sci Total Environ 571:206–217. https://doi.org/10.1016/j.scitotenv.2016.07.135
Zheng B, Zhu Y, Sardans J, Penuelas J, Su J (2018) QMEC: a tool for high-throughput quantitative assessment of microbial functional potential in C, N, P, and S biogeochemical cycling. Sci China Life Sci 61:1451–1462. https://doi.org/10.1007/s11427-018-9364-7
Zhou J, Gui H, Banfield CC, Wen Y, Zang H, Dippold MA, Charlton A, Jones DL (2021) The microplastisphere: Biodegradable microplastics addition alters soil microbial community structure and function. Soil Biol Biochem. https://doi.org/10.1016/j.soilbio.2021.108211
Zhu X, Mao L, Chen B (2019) Driving forces linking microbial community structure and functions to enhanced carbon stability in biochar-amended soil. Environ Int 133:105211. https://doi.org/10.1016/j.envint.2019.105211
Zimmerman AR, Gao B, Ahn M-Y (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol Biochem 43:1169–1179. https://doi.org/10.1016/j.soilbio.2011.02.005