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Polyethylene microplastics alter the microbial functional gene abundances and increase nitrous oxide emissions from paddy soils

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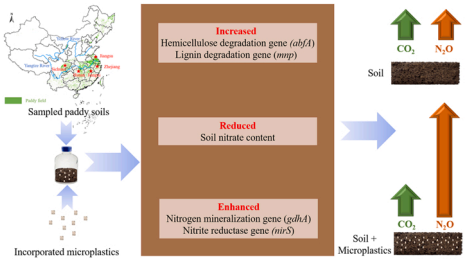
HIGHLIGHTS

- Polyethylene (PE) microplastic (MP) did not affect CO_2 emissions from paddy soils.
- PE MP addition substantially increased N_2O emission from paddy soils.
- PE MP increased the microbial functional genes involved in nitrite reductase (*nirS*).

GRAPHICAL ABSTRACT



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ABSTRACT

The accumulation of microplastics (MPs) in terrestrial ecosystems can affect greenhouse gases (GHGs) production by changing soil structure and microbial functions. In this study, microcosm experiments were conducted to investigate the impact of polyethylene (PE) MP addition on soil carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions from paddy soils and their associated microbial functional genes. Methane was not considered due to the negligible emissions throughout the incubation. The amendment of both virgin and aged PE MPs did not significantly ($p > 0.05$) affect soil CO_2 emissions, but significantly ($p < 0.05$) increased the abundances of microbial functional genes encoding enzymes involved in hemicellulose (*abfA*) and lignin (*mnp*) decomposition, indicating plastic particle has potential to stimulate soil organic carbon decomposition. The presence of PE MP significantly increased N_2O emissions by 3.7-fold, which was probably due to PE MP increased the abundances of *nirS* gene involved in nitrite reductase. In addition, compared with virgin PE MP treatment, artificially aged PE MP did not significantly ($p > 0.05$) influence soil CO_2 and N_2O emissions. Our results provide evidence that PE

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MP likely cause a high risk of N_2O emission from paddy soils, this factor should be considered in future estimates of GHGs emissions from rice fields.

1. Introduction

Microplastics (MPs), which are plastic particles (i.e., fragment, bead and fiber) less than 5 mm in size, indirectly originate from the fragmentation of plastic waste or are directly derived from primary small plastics (Law and Thompson, 2014; Thompson et al., 2004). These particles have been emerging pollutant because of their superior absorbability, and a hotspot is created around their surface that is enriched with organic or heavy metal contaminants (Law and Thompson, 2014; Thompson et al., 2004). It is predicted that 50 million metric tons of plastic waste will be discarded in terrestrial ecosystems by 2025 (MacLeod et al., 2021), which means that large amounts of MPs will be continuously incorporated into soils. Moreover, due to the resistant degradation of MPs (Rochman and Hoellein, 2020), it is expected that the increase in MP concentration will be maintained in the future. Agroecosystems are a major sink of MPs in terrestrial ecosystems, and the wide application of sewage sludge, plastic film, wastewater, organic fertilizer and plastic-coated fertilizer, or atmospheric deposition (Katsumi et al., 2021; Nizzetto et al., 2016; Wang et al., 2022; Zhang et al., 2020, 2021a) can input a large number of MPs into farmlands.

Over the past decade, researchers have shown an increase in interest in the distribution and environmental behavior of MPs in agricultural soils (Baho et al., 2021; Wang et al., 2022; Ya et al., 2021; Zhang et al., 2021a), but their effect on soil biogeochemical processes is still limited (Rillig and Lehmann, 2020). In general, the C component of nonbiodegradable MPs is difficult for microorganisms to utilize, and MPs contains negligible nitrogen (N) sources, which does not directly affect C and N biogeochemical cycling (Rillig and Lehmann, 2020). However, the presence of MPs can indirectly affect soil greenhouse gases (GHGs) emissions by changing soil structure (Rillig et al., 2021a; Wang et al., 2022). In general, the exposure of MPs can increase the availability of labile C in soils by inserting into soil macroaggregations (Zhang and Zhang, 2020) or improve soil aeration (Rillig et al., 2021a, 2021b), and thus stimulate microbial decomposition (Gao et al., 2021; Liu et al., 2017) or reduce N_2O production by inhibiting nitrite reduction in aerobic soils (Ren et al., 2020; Rillig et al., 2021a; Yu et al., 2021a). However, under saturated moisture conditions (e.g., paddy soils), the impact of MPs on soil CO_2 and N_2O emissions remain unclear.

In general, the water requirement of paddy fields is greater than that of dryland farmlands, and probably induces a large number of MPs coming into rice soils with irrigation waters. Zhou et al. (2020) reported that the abundance of MPs in irrigation water was up to 1.7×10^4 pieces m^{-3} in Hangzhou Bay, China, and contributed to the accumulation of MPs in agricultural soils. Wang et al. (2021) measured the MP concentrations in different agricultural fields of China and found that the abundances of MPs in paddy fields were close to those in plastic mulching soils, and were significantly greater than those in other non-mulched farmlands. Moreover, near the drainage outlet of paddy fields, large amounts of MPs accumulate and create a hotspot (Katsumi et al., 2021). Therefore, it is expected that the current MPs pollution has the potential to affect C and N dynamics in paddy soils.

Previous studies provided evidence that the impact of MPs on soil GHGs emissions in aerobic condition (upland soil) was different from saturated water condition (e.g., paddy soil). Based on a meta-analysis, Zhang et al. (2022) found that high dose (≥1.0%) of polyethylene (PE) MPs significantly increased soil CO_2 emissions, but low dose (≤0.1%) had negligible effects on SOC decomposition in upland soils. However, Xiao et al. (2021) found a contrast dose-dependent effect of PE MP on SOC decomposition in paddy soils. Previous publications found MPs can increase oxygen content of microenvironment, and then limit N_2O production through denitrification pathway in aerobic condition (Ren et al., 2020; Rillig et al., 2021a; Yu et al., 2021a). However, under flooded condition, Seeley et al. (2020) found that PE MPs increased the potential denitrification rates in sediments, which was probably beneficial for N_2O production. Until now, most of these studies have focused on the effect of MPs on GHGs emissions in upland soils (Brown et al., 2022; Gao et al., 2021; Ren et al., 2020; Rillig et al., 2021a; Rillig and Lehmann, 2020; Zhang et al., 2022), while the impact of this material on GHGs emissions in paddy soils remains unclear (Han et al., 2022; Xiao et al., 2022, 2021).

In agricultural fields, aged MPs exposed to ultraviolet (UV) radiation generally exhibit greater redox activity due to their high oxygen-containing functional groups and adsorption area (Li et al., 2020; Liu et al., 2019a, 2019b). According to the "electron shutting" hypothesis, if the aging process of MPs is similar to that of aged biochar, microbes prefer to use aged MPs as electron donors in soils and then likely stimulate SOC decomposition by improving energy efficiency during their metabolism (Rillig et al., 2021b); in addition, N_2O emissions are probably increased by inhibited N_2O reduction due to their low electron shuttle function (Yuan et al., 2019). However, until now, most studies have evaluated the impact of pristine MPs on soil GHGs emissions based on microcosm experiments, which likely underestimated the release of these gases after MPs amendment (Zhang et al., 2021b). In this experiment, five paddy soils were collected across southern of China, and microcosm experiments were performed to evaluate the impact of pristine and aged PE MPs on soil GHGs emissions and their associated microbial functional genes encoding enzymes involved in C and N biogeochemical cycling. We hypothesized that (1) the amendment of PE MPs accelerates SOC decomposition but increases N_2O emissions; and (2) the aging of PE MPs accelerates the release of CO_2 and N_2O emissions.

2. Materials and methods

2.1. Soil collection and experimental design

Five typical Stagnic Anthrosol paddy soils across southern China, including Yanting of Sichuan Province (SC, 105° 27′ E, 31° 16′ N), Changde of Hunan Province (HN, 111°41′ E, 29° 14′ N), Yingtan of Jiangxi Province (JX, 116° 55′ E, 28° 12′ N), Hangzhou of Zhejiang Province (ZJ, 120° 4′ E, 30° 5′N) and Yancheng of Jiangsu Province (JS, 120° 28' E, 32° 56' N) were collected (Fig. S1). These provinces are the main rice production regions in China, and the selected paddy fields have been cultivated for more than 15 years, which enables microorganisms to adapt to the waterlogged paddy soil environment (Cui et al., 2018). After the local rice harvest, soil samples in the 0–20 cm layer were manually collected, sieved through ≤ 2 mm mesh to remove rice residues or stones, and then stored at 4 °C for incubation experiments. Detailed information of these five paddy soils is shown in Table 1.

Three treatments, no MP addition (CK), 1% (w/w) virgin and 1% (w/w) aged PE MPs, with three replicates were applied to each paddy soil. The added amount of MP was environmentally relevant, as Sun et al. (2018) detected that the MP concentration was up to 1% in agricultural fields. PE MPs (< 187.5 μm) were used because this type of material is one of the major MPs in paddy fields (Katsumi et al., 2021). Most MPs in the soil environment age through weathering by solar radiation (Li et al., 2020). Therefore, the aged MPs was prepared as follows: virgin PE MPs were washed with deionized water, dried and then exposed to UV radiation (γmax=253.7 nm). During the aging process, the virgin MPs were placed into quartz glass vessels and mixed well every half day to ensure homogeneous exposure to radiation (Liu et al., 2019a). After seven days, field emission scanning electron microscopy (FESEM,

GeminiSEM300, ZEISS., Germany) and Fourier transform infrared (FTIR) spectroscopy (Nicolet 6700, Thermo Electron Corp., USA) were applied to detect the morphology and surface functional groups of MPs, respectively. In addition, the carbonyl index (*CI*) was used to indicate the aged degree of MPs using the equation $CI = P_c/P_m$, where P_c and P_m are the carbonyl peak at 1715 cm^{-1} and methylene scissoring peak at 1465 cm^{-1} (Albertsson et al., 1987; Liang et al., 2013). The *CI* value was higher for radiated MPs (0.42) than virgin MPs (0.28) (Fig. S2) and small fissures on the surfaces of UV-radiated MPs rather than virgin MPs (Fig. S3). These results indicated that virgin MPs were effectively aged by UV radiation (Liang et al., 2013).

2.2. Soil GHGs emissions measurements

Microcosm experiments were conducted to explore the effect of MPs on soil GHGs emissions, and four weeks was sufficient for both CO_2 and N_2O emissions remained at stable levels based on our preliminary experiment. PE MPs (1 g) were incorporated and then evenly mixed within 100 g each soil samples (dry weight) to achieve 1% (w/w). Afterwards, 15 g mixture (dry weight) was added to a 120 ml brown serum bottle and then incubated at 25 °C in a dark environment. In general, the paddy soil is characterized by anaerobic condition caused by flooding in paddy fields (Kögel-Knabner et al., 2010). However, due to the density of PE MPs (0.96 g cm^{-3}) is closed to the water density (1.0 g cm^{-3}), therefore, we adjusted the paddy soils to saturated water content to simulate anaerobic condition, and make ensure the 1% dose of PE MPs was accumulated in the soils.

Soil GHGs concentrations were measured every three days as follows: 1) the air renewal system of the fuming cupboard was used to refresh the inner gas concentrations in the bottles before each emissions measurement, and five minutes resulted in the GHGs content in the bottle becoming equal to the atmospheric background level (Fig. S4); 2) silicone plugs were applied to seal all jars and then incubated in paddy soils to produce GHGs; 3) after incubation for approximately 24 h, the inner gas of the bottles was sampled and then analyzed for GHGs concentrations; and 4) the bottles were opened for two days until the next measurement, meanwhile water loss was monitored by weighing the bottle every two days and added appropriate water to achieve a certain water content if necessary. A gas chromatographic instrument (Agilent 7890B, Agilent, Palo Alto, CA, USA) equipped with a hydrogen flame ionization detector (FID) was used to detect methane and CO_2 concentrations, and an electron capture detector (ECD) was applied to detect N_2O concentrations. Linear interpolation between daily GHGs emissions and corresponding time was used to compute the cumulative emissions during the whole experimental period.

2.3. Soil dissolved organic carbon (DOC) and mineral N measurements

All incubated soils were destructively sampled after 28 days. The DOC of the paddy soil was extracted by 0.5 M K_2SO_4 solution (3 g soil vs. 15 ml), shaken for 1 h, filtered by a quantitative filter (Jones and Willett, 2006), and then measured by a total organic carbon analyzer (Multi-N/C 2100 S; Analytik Jena, Jena, Germany). The ammonium (NH_4^+) and nitrate (NO_3^-) of rice soils were extracted via 2 M KCl (3 g soil vs. 15 ml), shaken for 60 min, filtered by a qualitative filter (Hood-Nowotny et al., 2010), and then analyzed via a continuous flow analyzer (Skalar SAN^{++} System, Skalar Analytical B.V., Breda, Netherlands).

2.4. DNA extraction and high-throughput quantitative PCR analysis

Five grams of soil was freeze-dried for more than two days at − 80 °C. Afterward, the DNA of the dried soil (0.5 g) was extracted using the FastDNATM Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the instruction manual. Afterwards, the quality of DNA was checked by a NanoDrop-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Based on the quantitative microbial element cycling (QMEC) checked by Zheng et al. (2018), 16 S rRNA (reference gene), 18 bacterial functional genes encoding enzymes involved in starch (*apu*, *sga, amyA*, *amyX* and *iso-plu*), hemicellulose (*xylA*, *abfA* and *manB*), cellulose (*naglu*, *cdh* and *cex*), chitin (*exo-chi* and *chiA*), pectin (*pgu*) and lignin (*mnp*, *pox*, *glx* and *lig*) hydrolyzes, and 13 microbial functional genes encoding enzymes related to N mineralization (*gdhA*), nitrification (*amoA1*, *amoA2*, *amoB*, *hao* and *nxrA*) and denitrification (*n arG* , *nirK1*, *nirK2*, *nirS1*, *nirS2*, *nosZ1* and *nosZ2*) were qualified by a high-throughput quantitative PCR-based chip (WaferGen Biosystems, Fremont, USA). The detailed information of primer pairs for these genes is shown in Table S1.

A 50 nL mixed reaction system (including primer (0.2 μmol L^{-1}), Premix Ex Taq (TaKaRa, 1 μL) and tenfold diluted DNA template (1 ng μL^{-1})) was used for amplification. The qPCR process was initial denaturation (10 min at 95 °C), 40 cycles (30 s at 95 °C), annealing (30 s at 58 °C) and extension (30 s at 72 °C) (Zheng et al., 2018). Every reaction for each primer set was performed in triplicate, and WaferGen software was applied to automatically generate the melting curve. After amplification, only the amplification efficiencies or multiple melting peaks ranged from 80% to 120% were selected, meanwhile, the threshold cycle (*Ct*) should be less than 31. The equation $R_{gene} = 10^{(31\text{-}Ct)/(10/3)}$ was used to estimate the relative copy number of each gene (including 16 S rRNA and microbial functional genes), and the ratio of R_{gene} and $R_{16S\ rRNA}$ was the normalized relative abundance of different microbial functional genes (N_{gene}) (Zheng et al., 2018). Finally, the absolute gene copies of the 16S rRNA gene ($A_{16S\ rRNA}$) was quantified by qPCR via LightCycler® 480 II (Roche Diagnostics, Basel, Switzerland) (Fig. S5), and then the equation $A_{gene} = A_{16S\ rRNA} \times N_{gene}$ was applied to calculate the absolute gene copy (A_{gene}) of each functional gene (Su et al., 2021).

2.5. Statistical analyses

The response ratio (*RR*) and its corresponding variance (v) were used to analyze the effect of MP addition on each variable across various soils (Hedges et al., 1999):

$$RR = ln\overline{M_{MPs}} - ln\overline{M_{CK}} \quad (1)$$

$$v = \frac{1}{3} \times \left(\frac{SD_{MPs}}{M_{MPs}}\right)^2 + \frac{1}{3} \times \left(\frac{SD_{CK}}{M_{CK}}\right)^2 \quad (2)$$

where $\overline{M_{MPs}}$ and $\overline{M_{CK}}$ are the mean values of each variable in the soils

Table 1
Site information and the properties of sampling soils. MAT: mean annual temperature; MAP: mean annual precipitation; SWC: saturated water content; SOC: soil organic carbon; TN: total nitrogen; C/N: the ratio of carbon and nitrogen.

Sites	MAT (℃)	MAP (mm)	SWC	pH	SOC (g kg^{-1})	TN (g kg^{-1})	C/N	Soil texture (%)		
			%					Clay	Silt	Sand
Sichuan	17.3	826	44.1	8.2	9.8	1.1	9.1	11	49	40
Hunan	16.5	1437	50.2	6.7	15.2	1.6	9.2	9	72	19
Jiangxi	17.6	1795	53.3	5.0	17.4	2.0	8.7	19	59	22
Zhejiang	17.5	1140	69.9	7.5	22.9	2.3	9.8	21	52	27
Jiangsu	15.0	1049	59.1	8.2	15.1	1.4	10.5	14	50	36

with and without MPs, respectively, and SD_{MPs} and SD_{CK} are the standard deviations of each variable in the soils with and without MPs, respectively. Afterward, a random-effects model of Stata 16.0 software (Stata Corporation, College Station, Texas, USA) was applied to calculate the weighted response ratio (RR_{++}) and the 95% confidence intervals (*CIs*) of RR_{++}, which were used to evaluate the effect of MP addition on soil GHGs emissions, soil properties (DOC, NH_4^+ and NO_3^-), microbial functional genes encoding enzymes involved in C and N cycling across the five soils. Moreover, the random-effect model was also used to detect the differences of these variables between the treatments with virgin and aged MPs. The effect of MP addition on these variables was considered significant at $p < 0.05$ if the 95% CI of RR_{++} did not overlap zero. Finally, the percentage change (P) of each variable induced by MP addition was calculated by $P = (exp^{RR_{++}} - 1) \times 100$ (Yu et al., 2021b).

One-way ANOVA was applied to evaluate the impact of MPs on soil GHGs emissions, soil properties (DOC, NH_4^+ and NO_3^-) and microbial functional genes by using the least significant differences (LSDs) test. All data are expressed as the mean ± standard deviation (SD). Meanwhile, a muti-factor ANOVA was conducted to evaluate the main and interactive effects of soil and MPs on soil GHGs emissions, properties and microbial functional genes. The relationship of *RRs* of soil GHGs emissions to MP addition with the *RRs* of soil properties (DOC, NH_4^+ and NO_3^-) or microbial functional genes to MPs were determined by correlation and regression analyses. All statistical analyses were performed by SPSS 20.0 statistical software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Cumulative GHGs emissions

Negligible methane emissions were observed for the all treatments (data not shown). All daily soil CO_2 emissions remained at relatively stable levels during the experimental period (Fig. S6). Cumulative CO_2 emissions ranged from 111.1 to 480.8 mg C kg^{-1} across all paddy soils (Fig. 1a). Soils and the interaction of soil and MPs addition significantly ($p < 0.05$) influenced cumulative CO_2 emissions (Table 2). Specifically, virgin and aged MPs significantly ($p < 0.05$) increased total CO_2 emissions by 15.8–36.1% and 10.0–14.4% for HN and JS soils, respectively, but did not significantly ($p > 0.05$) influence CO_2 emissions for the other soils. The total CO_2 emissions from the HN and JS soils without MPs were 111.1 ± 3.0 and 294.4 ± 2.3 mg C kg^{-1}, respectively; and the cumulative CO_2 emissions from the soils with virgin and aged PE MPs were 151.2 ± 1.0 and 128.7 ± 2.0 mg C kg^{-1} for HN soil and were 323.8 ± 10.3 and 336.9 ± 4.7 mg C kg^{-1} for JS soil, respectively. In addition, for ZJ soil, total CO_2 emissions amendment with aged MPs (368.7 ± 1.8 mg C kg^{-1}) were significantly ($p < 0.05$) higher than with virgin MPs (319.5 ± 3.5 mg C kg^{-1}), but an opposite result was found for HN soil.

Table 2
Main and interactive effects of soil and MPs on cumulative CO_2 and N_2O emissions.

Factors	Cumulative CO_2 emission	Cumulative N_2O emission
Soil	[a]	[a]
MPs	ns	[a]
Soil×MPs	[b]	[c]

[a] < 0.001
[b] < 0.05
[c] < 0.01

In the absence of MPs, the daily N_2O emissions maintained at relatively low rates (except for JX soil with low pH), but the addition of MPs enhanced the daily N_2O emissions for HN, JS and ZJ soils (Fig. S7). Cumulative N_2O emissions ranged from 16.3 to 1735.4 μg N kg^{-1} across all soils (Fig. 1b). Soils, MPs addition their interactions significantly ($p < 0.05$) influenced cumulative N_2O emissions (Table 2). The lowest and highest emissions were observed for SC and JX soils, respectively. Interestingly, the addition of MPs did not significantly ($p > 0.05$) influence N_2O emissions for these two soils. Both virgin and aged MPs largely increased total N_2O emissions by 13.6–41.9-, 3.5–9.5- and 8.9–16.8-fold for HN, ZJ and JS soils, respectively. In addition, for ZJ and JS soils, cumulative N_2O emissions amended with aged MPs were significantly ($p < 0.05$) higher than with virgin MPs; but the opposite result was found for HN soil. The total N_2O emissions from the HN, ZJ and JS soils without MPs were 35.7 ± 9.9, 114.2 ± 18.2 and 11.5 ± 2.1 mg N kg^{-1}, respectively; and the cumulative N_2O emissions from the soils with virgin and aged PE MPs were 1528.1 ± 170.5 and 519.0 ± 86.6 mg N kg^{-1} for HN soil, were 511.2 ± 110.2 and 1193.7.9 ± 162.6 mg N kg^{-1} for ZJ soil, and were 114.4 ± 12.8 and 205.4 ± 20.5 mg N kg^{-1} for JS soil, respectively.

3.2. Soil DOC and mineral N contents

The lowest (36.0–88.3 mg C kg^{-1}) and highest (190.5–211.6 mg C kg^{-1}) DOC contents were observed for JS and JX soils, respectively. Soils and the interaction of soils and MPs addition significantly ($p < 0.05$) affected soil DOC contents (Table 3). Both virgin and aged MPs increased (for SC soil), did not affect (for HN and JX soils) and reduced (for JS soil) DOC contents. In addition, virgin MPs did not influence DOC in ZJ soil but aged MPs significantly decreased DOC in ZJ soil. Soil NH_4^+ contents for the five paddy soils remained at relatively low levels (4.5–14.8 mg N kg^{-1}). Soils, MPs addition and their interactions significantly ($p < 0.05$) affected soil NO_3^- contents (Table 3). The lowest and highest soil NO_3^- contents were observed for SC

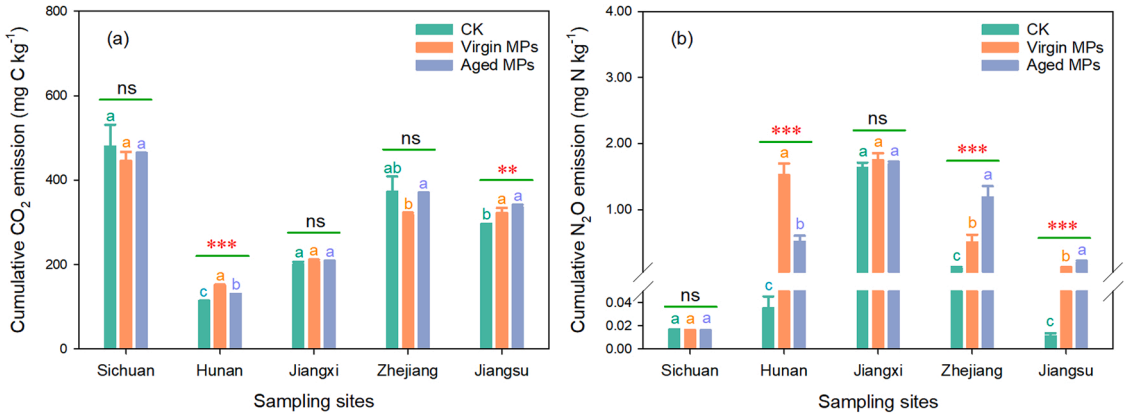

Fig. 1. Cumulative CO_2 (a) and N_2O (b) emissions from five paddy soils without (CK) or with virgin and aged PE MPs amendment treatments. The red asterisks indicate that MPs significantly affect soil CO_2 and N_2O emissions, and the different letters denote significant differences among the three treatments (t-test, 5%).

Table 3
Soil DOC, NH_4^+ and NO_3^- contents for the five paddy soils without (CK) or with virgin and aged PE MPs after incubation. The different letters denote significant differences among the three treatments (t-test, 5%). Statistical significance of the effects of the soil, MPs and their interactions on these variables (n = 3).

Sites	Treatments	DOC (mg C kg^{-1})	NH_4^+ (mg N kg^{-1})	NO_3^- (mg N kg^{-1})
Sichuan	CK	55.3 ± 12.8c	5.4 ± 0.8 b	4.9 ± 1.1 a
	Virgin MP	92.4 ± 8.5 b	9.3 ± 0.7 a	2.8 ± 0.1 b
	Aged MP	116.9 ± 3.0 a	9.1 ± 1.8 a	3.8 ± 0.7 ab
Hunan	CK	103.2 ± 11.4 a	8.7 ± 0.8 a	74.7 ± 2.4 a
	Virgin MP	159.7 ± 18.8 a	6.6 ± 0.7 b	60.3 ± 1.1 b
	Aged MP	128.9 ± 32.9 a	6.4 ± 0.5 b	77.7 ± 3.5 a
Jiangxi	CK	195.8 ± 1.9 a	14.8 ± 4.6 a	59.8 ± 3.8 a
	Virgin MP	211.6 ± 11.9 a	10.8 ± 4.2 a	52.7 ± 8.4 a
	Aged MP	190.5 ± 9.1 a	9.5 ± 1.7 a	52.0 ± 4.6 a
Zhejiang	CK	211.4 ± 4.5 a	6.1 ± 0.1 a	152.8 ± 16.3 a
	Virgin MP	217.1 ± 31.5 a	6.6 ± 0.4 a	154.4 ± 19.3 a
	Aged MP	131.0 ± 7.4 b	6.8 ± 0.4 a	161.3 ± 27.1 a
Jiangsu	CK	88.3 ± 14.6 a	4.8 ± 0.3 a	53.7 ± 1.4 a
	Virgin MP	60.6 ± 7.9 b	4.5 ± 0.1 a	8.2 ± 1.5c
	Aged MP	36.0 ± 6.2 b	4.7 ± 0.1 a	13.1 ± 1.6 b
ANOVA results				
Factors		DOC	NH_4^+	NO_3^-
Soil		* **	* **	* **
MPs		ns	ns	* *
Soil × MPs		* *	* *	* *

(2.8–4.9 mg N kg^{-1}) and ZJ (152.8–161.3 mg N kg^{-1}) soils, respectively. MP amendment reduced the NO_3^- content in SC and JS soils but did not affect it in JX and ZJ soils. In addition, virgin MPs reduced NO_3^-, whereas aged MPs did not influence NO_3^- in HN soil.

3.3. Microbial functional genes

Six groups of microbial functional genes encoding enzymes involved in the degradation of starch (*sga*), hemicellulose (*abfA*, *manB* and *xylA*) and lignin (*glx* and *mnp*) were detected (Fig. 2). Across the five soils, MPs addition only significantly ($p < 0.05$) affected *abfA* gene, and the interaction of soils and MPs significantly ($p < 0.05$) influenced *sga* and *xylA* genes (Table 4). For SC soil, there was no significant effect of MPs on the abundances of these genes (Fig. 2a). Compared with the CK treatment, both pristine and aged MPs significantly increased the abundances of *abfA* and *manB* genes in HN soil (Fig. 2b) and enhanced the abundances of *sga*, *abfA*, *glx* and *mnp* genes in JX soil (Fig. 2c). For ZJ soil, only aged MP addition increased the number of *abfA* gene after aged MP addition (Fig. 2d). For JS soil, both virgin and aged MPs significantly ($p < 0.05$) increased the abundance *abfA* gene, whereas virgin MPs promoted the abundances of *sga* and *xylA* genes compared to the CK treatment (Fig. 2e).

Six genes encoding enzymes related to N mineralization (*gdhA*), nitrification (*amoA*) and denitrification (*nirS1*, *nirS2*, *nosZ1* and *nosZ2*) were detected for the paddy soils (Fig. 3). Across the experimental soils, MPs addition only significantly ($p < 0.05$) affected *nirS1* gene, and the interaction of soils and MPs significantly ($p < 0.05$) influenced *amoA, nirS1, nirS2, nosZ1* and *nosZ2* genes (Table 4). There was no significant effect of MPs on the abundances of these genes in SC soil (Fig. 3a). MP amendment significantly ($p < 0.05$) increased *nirS* and *nosZ2* genes in HN soil (Fig. 3b) and *gdhA* and *amoA* genes in JX soil (Fig. 3c), but reduced *nosZ2* genes in ZJ soil (Fig. 3d). For JS soil, MP addition increased the abundances of *amoA*, *nirS1* and *nirS2* genes (Fig. 3e).

3.4. Effect of PE MPs on soil GHGs emissions, soil properties and microbial functional genes

Across the five paddy soils, compared with the CK treatment, MP addition did not significant ($p > 0.05$) influence cumulative CO_2 emissions (Fig. 4a), soil DOC and NH_4^+ contents (Fig. 4b), the abundances of *sga*, *manB*, *xylA* and *glx* genes (Fig. 4c) and the number of *amoA*, *nosZ1* and *nosZ2* genes (Fig. 4d); significantly ($p < 0.05$) increased total N_2O emissions by 3.7 fold (Fig. 4a), the abundances of *abfA* and *mnp* genes by 16.0% and 15.1%, respectively (Fig. 4c), and the number of *gdhA*, *nirS1* and *nirS2* genes by 10.5%, 24.7% and 17.1%, respectively (Fig. 4d); and significantly ($p < 0.05$) reduced soil NO_3^- content by 35.9% (Fig. 4b). In addition, compared with virgin PE MP treatment, aged PE MP just significantly increased soil NO_3^- content and *amoA* abundance by 23.7% and 10.3%, respectively (Fig. S8).

3.5. Relationships of soil CO_2/N_2O emissions with soil properties and microbial functional genes

There was no significant correlation between the response ratios of soil CO_2/N_2O emissions and the response ratios of soil DOC, NH_4^+ and NO_3^- contents (Fig. 5). Except for the *abfA* gene, there was no significant relationship between the response ratios of functional genes (*sga*, *manB*, *xylA*, *glx* and *mnp*) related to C degradation and the response ratio of soil CO_2 emissions to MP addition (Fig. 5a). In addition, except for the *nirS1* gene, there was no significant correlation between the response ratio of soil N_2O emissions to MP amendment and the response ratios of microbial functional genes encoding enzymes related to N mineralization (*gdhA*), nitrification (*amoA*) and denitrification (*nirS2*, *nosZ1* and *nosZ2*) (Fig. 5b).

After MP amendment, the change in microbial functional genes encoding enzymes involved in hemicellulose decomposition controlled the influence of MPs on soil CO_2 emissions, and there were positive linear relationships between the response ratios of soil CO_2 emissions to MP addition and the response ratios of *abfA* ($p < 0.05$) and *manB* ($p = 0.08$) genes at 95% and 90% confidence intervals, respectively (Fig. 6a). MP exposure increased soil N_2O emissions by controlling microbial functional genes encoding nitrite reductase, and there were positive linear correlations between the response ratios of soil N_2O emissions to MP amendment and the response ratios of *nirS1* ($p < 0.05$) and *nirS2* ($p = 0.09$) genes at 95% and 90% confidence intervals, respectively (Fig. 6b).

4. Discussion

4.1. Effect of MPs on soil CO_2 emission

Although the component of MPs is mostly carbon, this component is difficult for microorganisms to utilize and is expected to be stored in soils (Rillig, 2018; Rillig and Lehmann, 2020). The exposure of MPs can improve soil aeration by integrating into soil aggregates, enhance the activity of phenol oxidase to decompose resistant carbon, and then supply soluble organic C substrates for microbes to produce CO_2 during their metabolic processes (Gao et al., 2021; Liu et al., 2017; Ng et al., 2021; Rillig et al., 2021b; Tong et al., 2022). However, our experimental results indicated that no significant relationship was found between the change in DOC and CO_2 emissions after MP addition, indicating that the cumulative CO_2 emissions from paddy soils were likely not driven by soluble C substrates. Similar results were also reported by Zhang et al. (2022), who found that the changes in soluble C induced by MP addition did not regulate the CO_2 emissions from upland soils.

The presence of MPs can compete with soil microbes for physicochemical niches (Yu et al., 2020), which probably inhibits the expression of functional genes encoding enzymes related to organic C-degradation. Previous studies found that PE macroplastics or MPs reduced functional genes encoding enzymes involved in organic C degradation in upland soils and likely inhibited the ability of microorganisms to utilize C sources (Qian et al., 2018; Zhang et al., 2022). Qian et al. (2018) observed that residual PE plastic film reduced *β-glu* and *chi-A* genes encoding enzymes related to cellulose and chitin decomposition, and Zhang et al. (2022) found that PE MP addition decreased the functional genes encoding enzymes involved in starch (*sga*) and hemicellulose

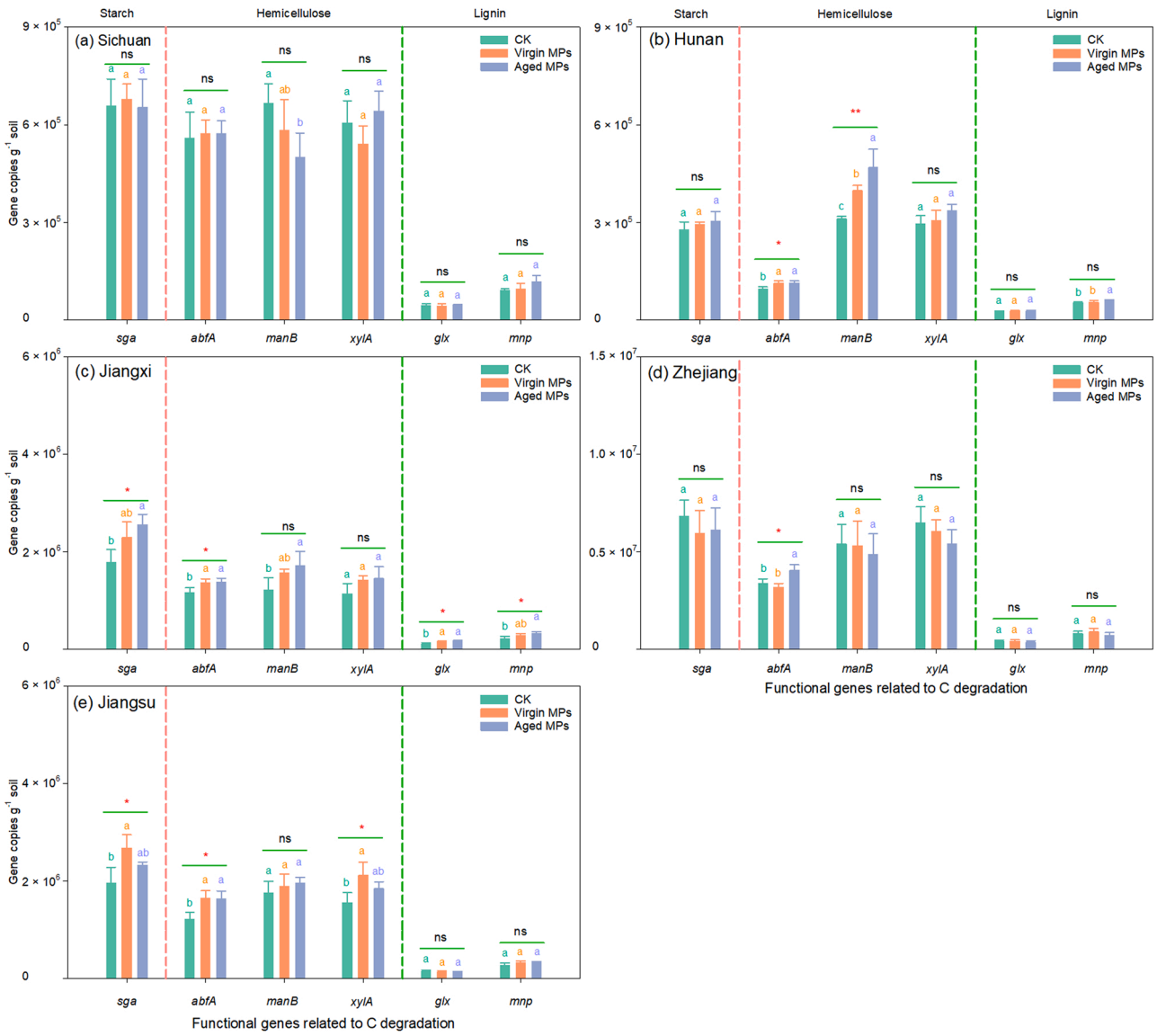

Fig. 2. Soil microbial functional genes encoding enzymes involved in starch (*sga*), hemicellulose (*abfA*, *manB* and *xylA*) and lignin (*glx* and *mnp*) decomposition in five paddy soils without (CK) or with virgin and aged PE MPs amendment treatments. The red asterisks indicate that MPs significantly affect soil microbial functional genes, and the different letters denote significant differences among the three treatments (t-test, 5%).

Table 4
Main and interactive effects of soil and MPs on functional genes encoding enzymes related to starch (*sga*), hemicellulose (*abfA*, *manB* and *xylA*) and ligin (*glx* and *mnp*) degradation, and N mineralization (*gdhA*), nitrification (*amoA*) and denitrification (*nirS1*, *nirS2*, *nosZ1* and *nosZ2*).

Factors	*sga*	*abfA*	*manB*	*xylA*	*glx*	*mnp*	*gdhA*	*amoA*	*nirS1*	*nirS2*	*nosZ1*	*nosZ2*
Soil	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]	[a]
MPs	ns	[b]	ns	ns	ns	ns	ns	ns	[c]	ns	ns	ns
Soil×MPs	[b]	ns	ns	[b]	ns	ns	ns	[b]	[c]	[c]	[c]	[c]

[a] < 0.001
[b] < 0.05
[c] < 0.01

(*abfA*, *manB* and *xylA*) hydrolyzes. However, Guo et al. (2021) detected that another type of MP, polyester microfibers, did not influence the microbial functional genes encoding enzymes related to organic C degradation. Our result was different with these publications, there was a linear relationship between the change in microbial functional genes encoding enzymes involved in hemicellulose degradation (*abfA* and *manB*) and soil CO_2 emissions, indicating that MP addition has the potential to accelerate the production of CO_2 from paddy soils by regulating the abilities of microbes to decompose labile C stocks.

Based on a meta-analysis method, Zhang et al. (2022) found a dose effect of MPs on the release of CO_2, as a high dose (≥1%) enhanced the production of CO_2 by 6.8%, whereas a low dose (≤0.1%) of this contaminant did not affect CO_2 emissions. However, Xiao et al. (2021) found a contrary dose-effect of PE MPs on SOC decomposition of paddy soils, as high dose (1%) of MPs did not significantly influence CO_2 emission, but low dose (0.01%) of MPs could accelerate SOC

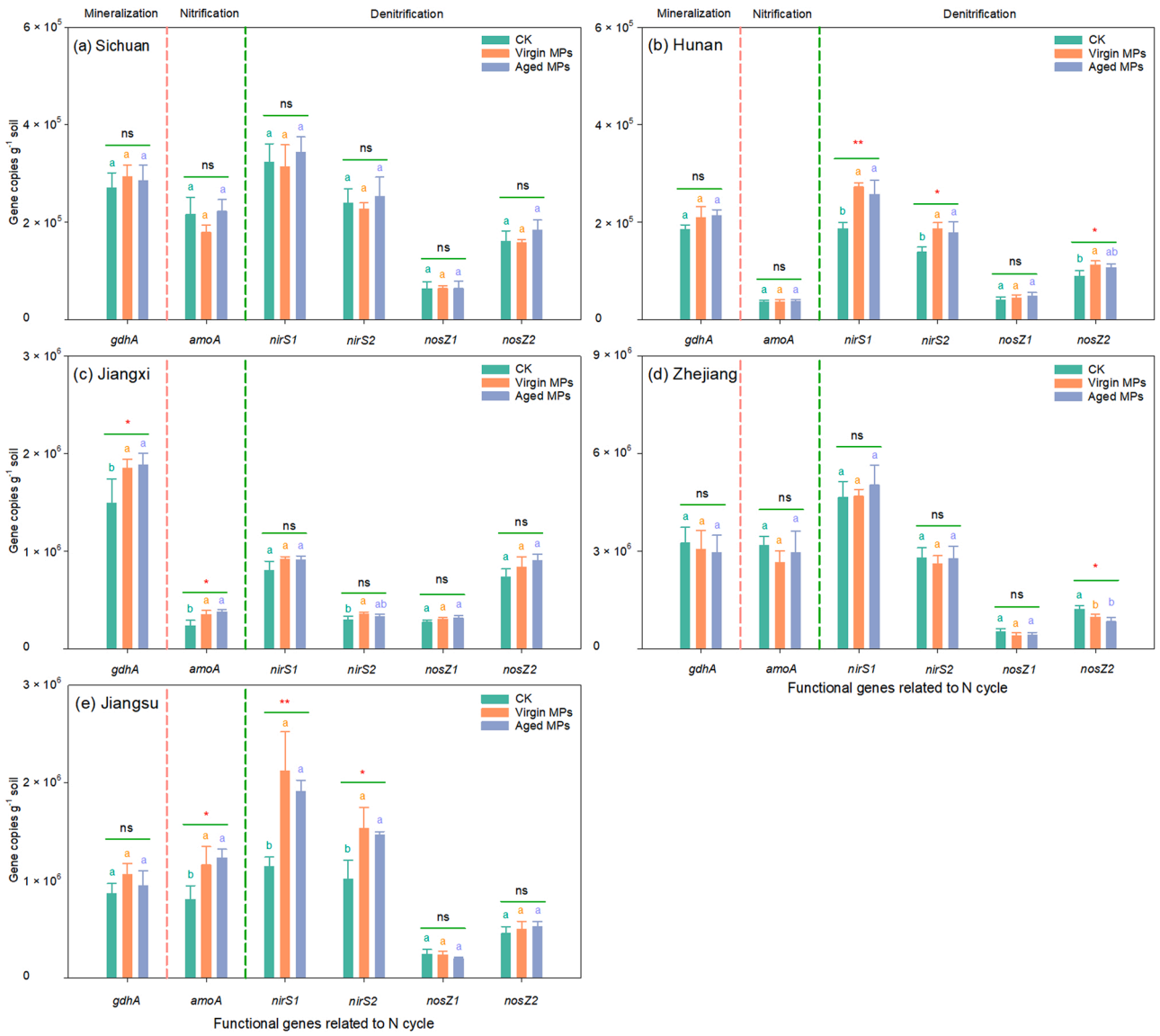

Fig. 3. Soil microbial functional genes encoding enzymes related to nitrogen mineralization (*gdhA*), nitrification (*amoA*) and denitrification (*nirS1*, *nirS2*, *nosZ1* and *nosZ2*) in five paddy soils without (CK) or with virgin and aged PE MPs amendment treatments. The red asterisks indicate that MPs significantly affect soil microbial functional genes, and the different letters denote significant differences among the three treatments (t-test, 5%).

decomposition by creating a suitable habitat for microorganisms. In the present experiment, 1% concentration of MPs significantly increased CO_2 emissions for the soils collected from HN and JS provinces but did not affect CO_2 emissions for the other soils. Interestingly, the SOC contents for HN and JS provinces were close to 15 g C kg^{-1}, which indicated that the impact of MPs on CO_2 production in paddy soils was probably dependent on SOC levels. Overall, across the five paddy soils, MPs addition did not significantly affect the release of CO_2 from paddy soils. This result was inconsistent with the impact of MPs on soil CO_2 production in unsaturated water condition (Gao et al., 2021; Rillig et al., 2021a; Zhang et al., 2022), because MPs increased the proportion of macropores and then enhanced mineralization process by improving oxygen supply (Rillig et al., 2021a). However, in this study, the experimental statured water condition blocked the gas exchange between atmosphere and soil macropores, and then limit the impact of MPs on CO_2 production through affecting soil structure.

4.2. Effect of MPs on soil N_2O emission

Previous studies have reported that PE MP amendment did not affect (Gao et al., 2021) or reduced (Ren et al., 2020; Rillig et al., 2021a; Yu et al., 2021a) N_2O emissions in upland soils. These differences were probably due to the impact of MPs on N_2O production or reduction pathways varying with soils. Gao et al. (2021) found that MPs addition increased the ammonium substrate for nitrification by enhancing N mineralization, but inhibited N_2O production by reducing the abundances of microbial functional genes encoding enzymes related to both nitrification (AOB *amoA*) and denitrification (*nirS*), and then had a negligible effect on N_2O emissions in vegetable soils. The reduction of N_2O emissions following MP amendment under aerobic condition was mainly due to:1) MP increase oxygen supply through improved soil aeration, and then inhibited the N_2O production through denitrification (Rillig et al., 2021a); 2) this material reduced the microbially available organic carbon through inhibited decomposition (Yu et al., 2021a) and then provided less energy for denitrifiers to produce N_2O (Lan et al., 2017).

In the present study, as expected, MP addition increased N_2O emissions across the five paddy soils, and there was a positive linear relationship between changes in N_2O emissions and *nirS* gene after MP addition. This result indicated that MPs probably enhance the nitrite

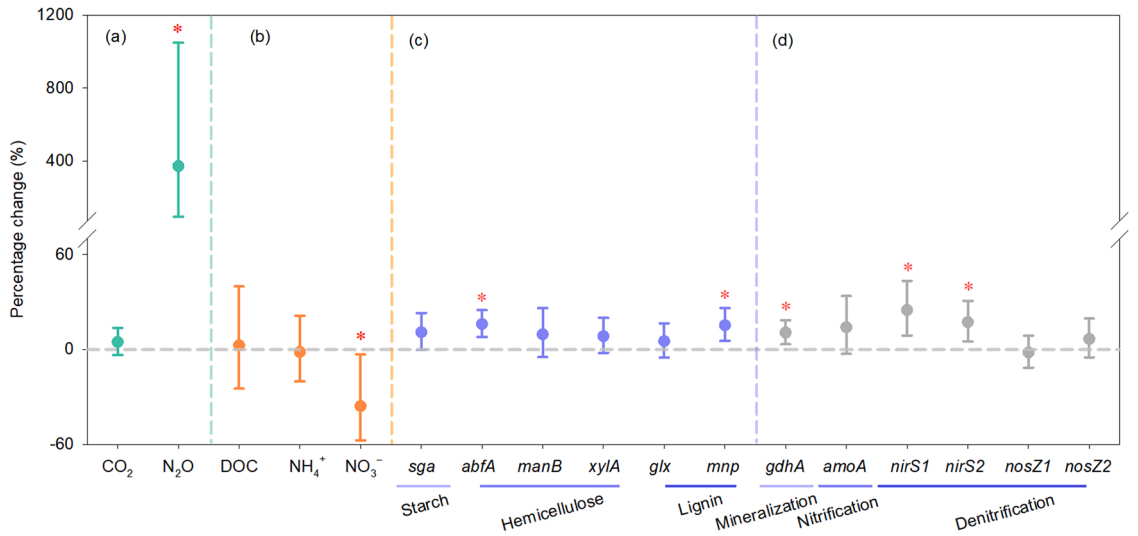

Fig. 4. The percentage changes in soil CO_2 and N_2O emissions (a), properties (DOC, NH_4^+ and NO_3^-) (b), microbial functional genes encoding enzymes involved in starch (*sga*), hemicellulose (*abfA*, *manB* and *xylA*) and lignin (*glx* and *mnp*) degradation (c) and nitrogen mineralization (*gdhA*), nitrification (*amoA*) and denitrification (*nirS1*, *nirS2*, *nosZ1* and *nosZ2*) (d) after PE MPs amendment. Red asterisks indicate MPs significantly affect these variables.

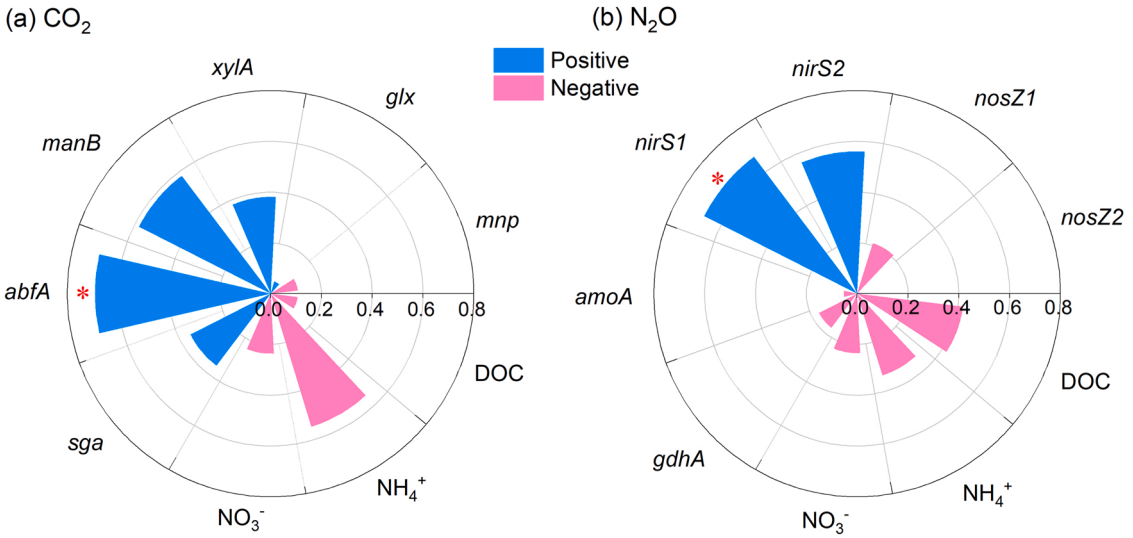

Fig. 5. Spearman correlation coefficients of the response ratio of soil CO_2 emissions to MP amendment with the response ratios of soil properties (DOC, NH_4^+ and NO_3^-) and microbial functional genes encoding enzymes involved in starch (*sga*), hemicellulose (*abfA*, *manB* and *xylA*) and lignin (*glx* and *mnp*) degradation (a), and the Spearman correlation coefficients of the response ratio of soil N_2O emissions to MPs amendment with the response ratios of soil properties (DOC, NH_4^+ and NO_3^-) and microbial functional genes encoding enzymes related to N mineralization (*gdhA*), nitrification (*amoA*) and denitrification (*nirS1*, *nirS2*, *nosZ1* and *nosZ2*) (b). Blue and pink colors indicate positive and negative correlation coefficients, respectively, and red asterisks indicate significant correlations. The response ratio was calculated with Eq. (1).

reduction process, and then increase N_2O production. This result is similar to that of Rong et al. (2021), who observed that 2% and 7% LDPE MPs increased the abundance of the functional gene (*nirK*) encoding nitrite reductase in upland soils, which probably increased N_2O production through denitrification. However, Ren et al. (2020) found the amendment of MP reducing N_2O formation by decreasing the abundance of *Chloroflexi* phylum which controls the *nirK* gene in the denitrification process. Yu et al. (2021a) observed that 10% PE MPs reduced N_2O emissions from unsaturated paddy soils, indicating that the impact of MPs on N_2O was also dependent on soil moisture. Notably, in this study, the functional genes encoding nitrite reductase (*nirS*) differed with Rong et al. (2021), which was probably because the activity of the *nirS* gene in paddy soils was greater than that of the *nirK* gene under anaerobic conditions and played an important role in controlling N_2O production (Liang et al., 2021). In another experiment, Han et al. (2022) found that the addition of polyethylene terephthalate MP increased the abundance of nitrification genes (*amoA* and *amoB*), but this material hardly affected N_2O emissions because of the lower contribution of nitrification to N_2O emissions than that of denitrification in paddy soils. Therefore, further research should focus on the relative contribution of nitrification and denitrification to N_2O emissions under different water conditions.

The impact of MPs on N_2O emissions varied with paddy soils, and this result was probably due to the low mineral N content or pH limiting the impact of MPs on N_2O emissions. In this study, the experimental soils with low mineral N content (collected from SC province) limited the effect of MPs on microbial functional genes encoding enzymes involved in both nitrification and denitrification, and then had no significant change in N_2O emissions following MP addition. For the acid soil (pH=5.0) sampled from JX Province, MP addition increased the abundance of *amoA* gene, which has the potential to increase N_2O production

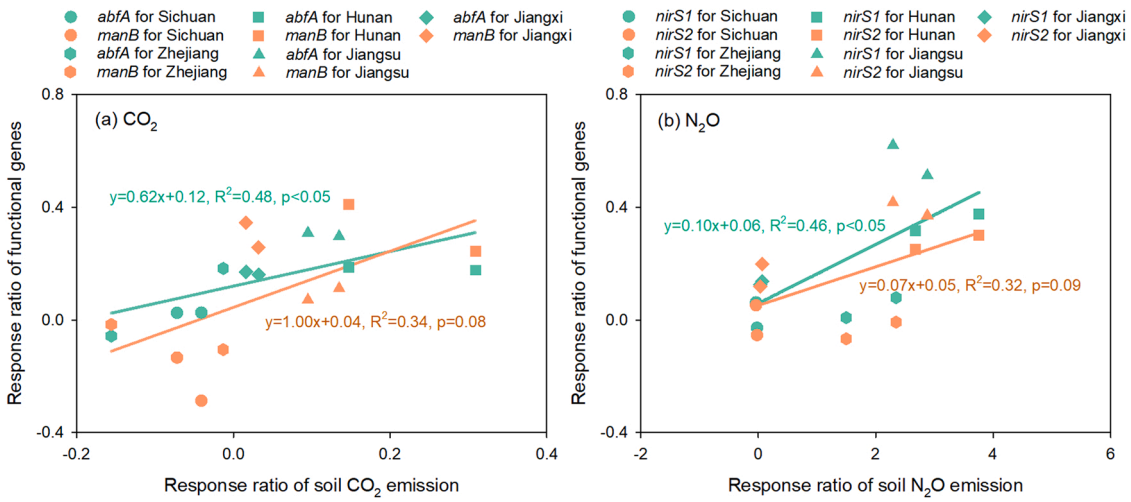

Fig. 6. Linear regression relationship between the response ratio of soil CO_2 emissions to MP addition and the response ratio of microbial function genes encoding enzymes related to C degradation (*abfA* and *manB*) (a), and the relationship between the response ratio of soil N_2O emissions to the response ratio of microbial function genes encoding enzymes related to N_2O production by denitrification (*nirS1* and *nirS2*) (b).

through nitrification. However, Ha et al. (2015) reported low pH ($\leq$ 5.8) inhibited the activity of N_2O reductase (*nosZ*) and caused higher N_2O emissions, which probably reduced the positive effect of MPs on N_2O emissions by enhancing nitrification. For the other soils, the increase in N_2O emissions after MP addition also varied with different pathways. For the paddy soils sampled from HN and JS provinces, MP amendment increased the abundance of *nirS* gene-encoded nitrite reductase and then induced higher N_2O production. However, for the soils collected from ZJ Province, MP addition reduced the gene copies of *nosZ* and then increased N_2O emissions by inhibiting N_2O reduction.

The intrinsic carbon of MPs can hardly supply an energy source for the production of N_2O during the nitrate reduction processes by denitrifiers (Lan et al., 2017). Yu et al. (2020) found that PE MPs can reduce aggregated organic C contents under aerobic condition by breaking their physical protection, and then probably indirectly influence N_2O production by increasing the soluble organic C contents. In this experiment, MPs addition increased N_2O emissions, but did not affect or even reduced DOC contents, indicating that the impact of MPs on N_2O emissions through the denitrification process was likely not regulated by labile C substrates in paddy soils. In addition, MPs contain minor N substrates, but the direct impact of this substrate on N_2O production has not been documented yet. However, MPs can potentially reduce nitrate by enhancing soil aeration and then decreasing N_2O production during denitrification (Rillig and Lehmann, 2020). Zhang et al. (2022) found that increasing PE MP concentrations from 0.01% to 1.0% continuously reduced NO_3^- contents of upland soil under aerobic condition, which means that this material has the potential to increase N_2O production with the exhaustion of NO_3^- in soils. However, some studies reported that PE MPs had no significant effect on soil mineral N contents (Blöcker et al., 2020; Meng et al., 2021) and probably did not affect N_2O production by regulating the availability of N substrates. In this study, the presence of MPs reduced NO_3^- contents and increased N_2O emissions for the soil collected from JS Province, which indicated that the effect of MPs on N_2O production by regulating NO_3^- substrates varied with paddy soils.

4.3. Effect of aged MPs on soil CO_2 and N_2O emissions

The surface of aged MPs generally has higher oxygen-containing functional groups and adsorption areas, which would attract microbes to prefer to utilize them as electron donors (Rillig et al., 2021b). Therefore, compared with virgin MPs, the aging of MPs will increase CO_2 and N_2O emissions by improving the metabolic energy efficiency of microbes (Rillig et al., 2021b) and probably inhibiting N_2O reduction during the denitrification process (Yuan et al., 2019), respectively. However, in the present study, across the five paddy soils, compared with the soil amendment with pristine MPs, the artificially aged MPs did not significantly affect soil CO_2 and N_2O emissions. A possible explanation may be that the aging degree of MPs was lower than that of MP particles after long-term weathering or UV radiation exposure in the fields (Li et al., 2020). Therefore, the presence of MPs in the fields should be extracted and then incorporated into experimental paddy soils, and then applied to evaluate the impact of a high degree of aging MPs on N_2O emissions in the future. In addition, compared with virgin PE MP treatment, artificially aged PE MP significantly increased soil NO_3^- content and *amoA* abundance, indicating aged PE likely increase nitrification rate and then support more substrate for denitrification, which has potential to improve the release of N_2O from paddy soils.

4.4. Limitations of this study

Our results provided evidence that the presence of MP addition probably stimulated the N_2O production from paddy soils. However, in the present study, three limitations should be noted. First, previous studies found shape, size and concentration of MPs are the important factors controlling soil properties (Lozano et al., 2021; Qi et al., 2020; Wang et al., 2022). Accordingly, the impact of PE MP on GHG emissions from paddy soils probably varied with the traits of MP. Second, it is worth noting that MPs addition increase N_2O emission during the 4 weeks after MPs addition, however, this period is probably too short to the functional time for paddy soil (Kögel-Knabner et al., 2010). Further long-term incubation it is crucial to explore the different properties of MPs on soil biogeochemistry process of paddy soils. Third, the impact of MPs on N_2O emissions varied with paddy soils was likely attributed to the different water content among various soils, because the N_2O production through nitrification and denitrification processes depending soil moisture (Bateman and Baggs, 2005). In addition, paddy soil is a major source of the atmospheric methane, but we detected negligible methane emission throughout the incubation, which was probably due to the lack of additional carbon sources (such as rice straw or exudates) for methanogenesis (Kögel-Knabner et al., 2010). Therefore, additional carbon sources should be incorporated into paddy soils to investigate the impact of MPs on methane emission.

5. Conclusion

Our results highlighted the potential impact of PE MPs on CO_2 and N_2O emissions from paddy soils. Across the five paddy soils, the amendment of MPs insignificantly increased CO_2 release from soil. However, MPs addition significantly increased the microbial functional genes encoding enzymes related to hemicellulose (*abfA*) and lignin (*mnp*) degradation by 16.0% and 15.1%, respectively, and then has the potential to accelerate soil organic carbon decomposition. The presence of PE MPs was found to increase the functional gene (*nirS*) associated with nitrite reductase under denitrification. This might explain the significant increase of N_2O emission across paddy soils. Overall, our results provided evidence that MP addition likely accelerated the release of N_2O emissions from paddy soils and then offset the climate mitigation benefit by improved SOC storage.

CRediT authorship contribution statement

Yu Yongxiang, Li Xing: Conceptualization, Methodology, Software, Data Curation, Validation, Writing – original draft, Visualization, Investigation, Writing – review & editing, Supervision, Funding acquisition. **Feng Ziyi:** Methodology, Data Curation, Validation. **Xiao Mouliang, Ge Tida, Li Yaying:** Writing – review & editing. **Yao Huaiying:** Conceptualization, Data Curation, Validation, Visualization, Investigation, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.128721.

References

Albertsson, A.-C., Andersson, S.O., Karlsson, S., 1987. The mechanism of biodegradation of polyethylene. Polym. Degrad. Stabil. 18, 73–87.

Baho, D.L., Bundschuh, M., Futter, M.N., 2021. Microplastics in terrestrial ecosystems: moving beyond the state of the art to minimize the risk of ecological surprise. Glob. Change Biol. 27 (17), 3969–3986. https://doi.org/10.1111/gcb.15724.

Bateman, E.J., Baggs, E.M., 2005. Contributions of nitrification and denitrification to N_2O emissions from soils at different water-filled pore space. Biol. Fertil. Soils 41 (6), 379–388. https://doi.org/10.1007/s00374-005-0858-3.

Blöcker, L., Watson, C., Wichern, F., 2020. Living in the plastic age - different short-term microbial response to microplastics addition to arable soils with contrasting soil organic matter content and farm management legacy. Environ. Pollut. 267, 115468 https://doi.org/10.1016/j.envpol.2020.115468.

Brown, R.W., Chadwick, D.R., Thornton, H., Marshall, M.R., Bei, S., Distaso, M.A., Bargiela, R., Marsden, K.A., Clode, P.L., Murphy, D.V., Pagella, S., Jones, D.L., 2022. Field application of pure polyethylene microplastic has no significant short-term effect on soil biological quality and function. Soil Biol. Biochem. 165, 108496 https://doi.org/10.1016/j.soilbio.2021.108496.

Cui, J., Li, Y., Wang, C., Kim, K.S., Wang, T., Liu, S., 2018. Characteristics of the rhizosphere bacterial community across different cultivation years in saline-alkaline paddy soils of Songnen Plain of China. Can. J. Microbiol. 64 (12), 925–936. https://doi.org/10.1139/cjm-2017-0752.

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 (2), 352–365. https://doi.org/10.1002/etc.4916.

Guo, Q.Q., Xiao, M.R., Zhang, G.S., 2021. The persistent impacts of polyester microfibers on soil bio-physical properties following thermal treatment. J. Hazard. Mater. 420, 126671 https://doi.org/10.1016/j.jhazmat.2021.126671.

Ha, T.K.T., Maeda, M., Fujiwara, T., Nagare, H., Akao, S., 2015. Effects of soil type and nitrate concentration on denitrification products (N_2O and N_2) under flooded conditions in laboratory microcosms. Soil Sci. Plant Nutr. 61 (6), 999–1004. https://doi.org/10.1080/00380768.2015.1094747.

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 (Pt B), 118386 https://doi.org/10.1016/j.envpol.2021.118386.

Hedges, L.V., Gurevitch, J., Curtis, P.S., 1999. The meta-analysis of response ratios in experimental ecology. Ecology 80 (4), 1150–1156.

Hood-Nowotny, R., Umana, N.H.-N., Inselbacher, E., Oswald- Lachouani, P., Wanek, W., 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Sci. Soc. Am. J. 74 (3), 1018–1027. https://doi.org/10.2136/sssaj2009.0389.

Jones, D., Willett, V., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol. Biochem. 38 (5), 991–999. https://doi.org/10.1016/j.soilbio.2005.08.012.

Katsumi, N., Kusube, T., Nagao, S., Okochi, H., 2021. Accumulation of microcapsules derived from coated fertilizer in paddy fields. Chemosphere 267, 129185. https://doi.org/10.1016/j.chemosphere.2020.129185.

Kögel-Knabner, I., Amelung, W., Cao, Z., Fiedler, S., Frenzel, P., Jahn, R., Kalbitz, K., Kölbl, A., Schloter, M., 2010. Biogeochemistry of paddy soils. Geoderma 157 (1–2), 1–14. https://doi.org/10.1016/j.geoderma.2010.03.009.

Lan, Z.M., Chen, C.R., Rashti, M.R., Yang, H., Zhang, D.K., 2017. Stoichiometric ratio of dissolved organic carbon to nitrate regulates nitrous oxide emission from the biochar-amended soils. Sci. Total Environ. 576, 559–571. https://doi.org/10.1016/j.scitotenv.2016.10.119.

Law, K.L., Thompson, R.C., 2014. Microplastics in the seas. Science 345 (6193), 144–145. https://doi.org/10.1002/2014ef000240/.

Li, W., Wufuer, R., Duo, J., Wang, S., Luo, Y., Zhang, D., Pan, X., 2020. Microplastics in agricultural soils: extraction and characterization after different periods of polythene film mulching in an arid region. Sci. Total Environ. 749, 141420 https://doi.org/10.1016/j.scitotenv.2020.141420.

Liang, W., Luo, Y., Song, S., Dong, X., Yu, X., 2013. High photocatalytic degradation activity of polyethylene containing polyacrylamide grafted TiO_2. Polym. Degrad. Stabil. 98, 1754–1761. https://doi.org/10.1016/j.polymdegradstab.2013.05.027.

Liang, Y., Wu, C., Wei, X., Liu, Y., Chen, X., Qin, H., Wu, J., Su, Y., Ge, T., Hu, Y., 2021. Characterization of *nirS*- and *nirK*-containing communities and potential denitrification activity in paddy soil from eastern China. Agric. Ecosyst. Environ. 319, 107561 https://doi.org/10.1016/j.agee.2021.107561.

Liu, G., Zhu, Z., Yang, Y., Sun, Y., Yu, F., Ma, J., 2019a. Sorption behavior and mechanism of hydrophilic organic chemicals to virgin and aged microplastics in freshwater and seawater. Environ. Pollut. 246, 26–33. https://doi.org/10.1016/j.envpol.2018.11.100.

Liu, H., Yang, X., Liu, G., Liang, C., Xue, S., Chen, H., Ritsema, C.J., Geissen, V., 2017. Response of soil dissolved organic matter to microplastic addition in Chinese loess soil. Chemosphere 185, 907–917. https://doi.org/10.1016/j.chemosphere.2017.07.064.

Liu, P., Qian, L., Wang, H., Zhan, X., Lu, K., Gu, C., Gao, S., 2019b. New insights into the aging behavior of microplastics accelerated by advanced oxidation processes. Environ. Sci. Technol. 53 (7), 3579–3588. https://doi.org/10.1021/acs.est.9b00493.

Lozano, Y.M., Lehnert, T., Linck, L.T., Lehmann, A., Rillig, M.C., 2021. 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.

MacLeod, M., Arp, H.P.H., Tekman, M.B., Jahnke, A., 2021. The global threat from plastic pollution. Science 373, 61–65.

Meng, F., Yang, X., Riksen, M., Geissen, V., 2021. Effect of different polymers of microplastics on soil organic carbon and nitrogen - a mesocosm experiment. Environ. Res 204 (Pt A), 111938. https://doi.org/10.1016/j.envres.2021.111938.

Ng, E.L., Lin, S.Y., Dungan, A.M., Colwell, J.M., Ede, S., Huerta Lwanga, E., Meng, K., Geissen, V., Blackall, L.L., Chen, D., 2021. Microplastic pollution alters forest soil microbiome. J. Hazard. Mater. 409, 124606 https://doi.org/10.1016/j.jhazmat.2020.124606.

Nizzetto, L., Futter, M., Langaas, S., 2016. Are agricultural soils dumps for microplastics of urban origin? Environ. Sci. Technol. 50 (20), 10777–10779. https://doi.org/10.1021/acs.est.6b04140.

Qi, Y., Beriot, N., Gort, G., Huerta Lwanga, E., Gooren, H., Yang, X., Geissen, V., 2020. Impact of plastic mulch film debris on soil physicochemical and hydrological properties. Environ. Pollut. 266 (Pt 3), 115097 https://doi.org/10.1016/j.envpol.2020.115097.

Qian, H., Zhang, M., Liu, G., Lu, T., Qu, Q., Du, B., Pan, X., 2018. Effects of soil residual plastic film on soil microbial community structure and fertility. Water Air Soil Pollut. 229 (8), 261. https://doi.org/10.1007/s11270-018-3916-9.

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, M.C., 2018. Microplastic disguising as soil carbon storage. Environ. Sci. Technol. 52 (11), 6079–6080. https://doi.org/10.1021/acs.est.8b02338.

Rillig, M.C., Hoffmann, M., Lehmann, A., Liang, Y., Lück, M., Augustin, J., 2021a. Microplastic fibers affect dynamics and intensity of CO_2 and N_2O fluxes from soil

differently. Micro Nanoplast. 1 (1), 3. https://doi.org/10.1186/s43591-021-00004-0.
Rillig, M.C., Lehmann, A., 2020. Microplastic in terrestrial ecosystems. Science 368 (6498), 1430–1431.
Rillig, M.C., Leifheit, E., Lehmann, J., 2021b. Microplastic effects on carbon cycling processes in soils. PLoS. Biol. 19 (3), e3001130 https://doi.org/10.1371/journal.pbio.3001130.g001.
Rochman, C.M., Hoellein, T., 2020. The global odyssey of plastic pollution. Science 368, 1184–1185.
Rong, L., Zhao, L., Zhao, L., Cheng, Z., Yao, Y., Yuan, C., Wang, L., Sun, H., 2021. LDPE microplastics affect soil microbial communities and nitrogen cycling. Sci. Total Environ. 773, 145640 https://doi.org/10.1016/j.scitotenv.2021.145640.
Seeley, M.E., Song, B., Passie, R., Hale, R.C., 2020. Microplastics affect sedimentary microbial communities and nitrogen cycling. Nat. Commun. 11 (1), 2372. https://doi.org/10.1038/s41467-020-16235-3.
Su, X., Li, G., Cotner, J.B., Wei, L., Wang, Y., Pan, T., Ding, K., 2021. Long-term organic fertilization changes soil active bacterial composition and multifunctionality: RNA-based bacterial community and qPCR-based SmartChip analysis. J. Soils Sediment. 21 (2), 799–809. https://doi.org/10.1007/s11368-020-02854-2.
Sun, M., Ye, M., Jiao, W., Feng, Y., Yu, P., Liu, M., Jiao, J., He, X., Liu, K., Zhao, Y., Wu, J., Jiang, X., Hu, F., 2018. Changes in tetracycline partitioning and bacteria/phage-comediated ARGs in microplastic-contaminated greenhouse soil facilitated by sophorolipid. J. Hazard. Mater. 345, 131–139. https://doi.org/10.1016/j.jhazmat.2017.11.036.
Thompson, R.C., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W., McGonigle, D., Russell, A.E., 2004. Lost at sea: where is all the plastic? Science 304 (5672), 838. https://doi.org/10.1126/science.1094559.
Tong, Y., Ding, J., Xiao, M., Shahbaz, M., Zhu, Z., Chen, M., Kuzyakov, Y., Deng, Y., Chen, J., Ge, T., 2022. Microplastics affect activity and spatial distribution of C, N, and P hydrolases in rice rhizosphere. Soil Ecol. Lett. https://doi.org/10.1007/s42832-022-0138-2.
Wang, F., Wang, Q., Adams, C.A., Sun, Y., Zhang, S., 2022. Effects of microplastics on soil properties: current knowledge and future perspectives. J. Hazard. Mater. 424 (Pt C), 127531 https://doi.org/10.1016/j.jhazmat.2021.127531.
Wang, J., Li, J., Liu, S., Li, H., Chen, X., Peng, C., Zhang, P., Liu, X., 2021. Distinct microplastic distributions in soils of different land-use types: a case study of Chinese farmlands. Environ. Pollut. 269, 116199 https://doi.org/10.1016/j.envpol.2020.116199.
Xiao, M., Ding, J., Luo, Y., Zhang, H., Yu, Y., Yao, H., Zhu, Z., Chadwick, D.R., Jones, D., Chen, J., Ge, T., 2022. Microplastics shape microbial communities affecting soil organic matter decomposition in paddy soil. J. Hazard. Mater. 431, 128589 https://doi.org/10.1016/j.jhazmat.2022.128589.
Xiao, M., Shahbaz, M., Liang, Y., Yang, J., Wang, S., Chadwicka, D.R., Jones, D., Chen, J., Ge, T., 2021. Effect of microplastics on organic matter decomposition in paddy soil amended with crop residues and labile C: a three-source-partitioning study. J. Hazard. Mater. 416, 126221 https://doi.org/10.1016/j.jhazmat.2021.126221.
Ya, H., Jiang, B., Xing, Y., Zhang, T., Lv, M., Wang, X., 2021. Recent advances on ecological effects of microplastics on soil environment. Sci. Total Environ. 798, 149338 https://doi.org/10.1016/j.scitotenv.2021.149338.
Yu, H., Fan, P., Hou, J., Dang, Q., Cui, D., Xi, B., Tan, W., 2020. Inhibitory effect of microplastics on soil extracellular enzymatic activities by changing soil properties and direct adsorption: an investigation at the aggregate-fraction level. Environ. Pollut. 267, 115544 https://doi.org/10.1016/j.envpol.2020.115544.
Yu, H., Zhang, Z., Zhang, Y., Song, Q., Fan, P., Xi, B., Tan, W., 2021a. Effects of microplastics on soil organic carbon and greenhouse gas emissions in the context of straw incorporation: a comparison with different types of soil. Environ. Pollut. 288, 117733 https://doi.org/10.1016/j.envpol.2021.117733.
Yu, Y., Zhang, Y., Xiao, M., Zhao, C., Yao, H., 2021b. A meta-analysis of film mulching cultivation effects on soil organic carbon and soil greenhouse gas fluxes. Catena 206, 105483. https://doi.org/10.1016/j.catena.2021.105483.
Yuan, H., Zhang, Z., Li, M., Clough, T., Wrage-Mönnig, N., Qin, S., Ge, T., Liao, H., Zhou, S., 2019. Biochar's role as an electron shuttle for mediating soil N_2O emissions. Soil Biol. Biochem. 133, 94–96. https://doi.org/10.1016/j.soilbio.2019.03.002.
Zhang, B., Yang, X., Chen, L., Chao, J., Teng, J., Wang, Q., 2020. Microplastics in soils: a review of possible sources, analytical methods and ecological impacts. J. Chem. Technol. Biotechnol. 95 (8), 2052–2068. https://doi.org/10.1002/jctb.6334.
Zhang, G.S., Zhang, F.X., 2020. Variations in aggregate-associated organic carbon and polyester microfibers resulting from polyester microfibers addition in a clayey soil. Environ. Pollut. 258, 113716 https://doi.org/10.1016/j.envpol.2019.113716.
Zhang, S., Wang, J., Yan, P., Hao, X., Xu, B., Wang, W., Aurangzeib, M., 2021a. Non-biodegradable microplastics in soils: a brief review and challenge. J. Hazard. Mater. 409, 124525 https://doi.org/10.1016/j.jhazmat.2020.124525.
Zhang, X., Li, Y., Ouyang, D., Lei, J., Tan, Q., Xie, L., Li, Z., Liu, T., Xiao, Y., Farooq, T.H., Wu, X., Chen, L., Yan, W., 2021b. Systematical review of interactions between microplastics and microorganisms in the soil environment. J. Hazard. Mater. 418, 126288 https://doi.org/10.1016/j.jhazmat.2021.126288.
Zhang, Y., Li, X., Xiao, M., Feng, Z., Yu, Y., Yao, H., 2022. Effects of microplastics on soil carbon dioxide emissions and the microbial functional genes involved in organic carbon decomposition in agricultural soil. Sci. Total Environ. 806 (Pt 3), 150714 https://doi.org/10.1016/j.scitotenv.2021.150714.
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. Chin. Life Sci. 61 (12), 1451–1462. https://doi.org/10.1007/s11427-018-9364-7.
Zhou, B., Wang, J., Zhang, H., Shi, H., Fei, Y., Huang, S., Tong, Y., Wen, D., Luo, Y., Barcelo, D., 2020. Microplastics in agricultural soils on the coastal plain of Hangzhou Bay, East China: multiple sources other than plastic mulching film. J. Hazard. Mater. 388, 121814 https://doi.org/10.1016/j.jhazmat.2019.121814.