

Research Paper

Microplastics stimulated nitrous oxide emissions primarily through denitrification: A meta-analysis

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HIGHLIGHTS

- Soil emitted 140.6 % more N₂O at exposure to microplastics.
- Denitrification rate was increased by microplastics rather than nitrification rate.
- The copies of denitrifiers were increased by 10.6 %.
- N₂O emissions increased more dramatically in the short term.

GRAPHICAL ABSTRACT



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ABSTRACT

Microplastics can profoundly alter nitrogen cycling. However, it remains poorly understood how microplastics impact soil nitrogen processes and generate N₂O. A meta-analysis was conducted for this investigation based on 60 published studies to elucidate the effects of microplastics on soil nitrogen cycling, from genes to processes. Under microplastic exposure, the emissions of soil N₂O was significantly increased (140.6%), while the nitrate reductase activities increased by 4.8%. The denitrification rate and number of denitrifier genes were increased by 17.8% and 10.6%, respectively. Meanwhile, the nitrification rate and nitrifier genes were not significantly altered, so did the nitrogen immobilization and mineralization rates. The additional emission of soil N₂O might primarily from stimulated denitrification. Soil N₂O emission and denitrification genes were always increased,

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regardless of the concentrations of microplastic or experiment duration. As a result, the nitrite was increased by 38.8% and nitrate was decreased by 22.4%, respectively. Interestingly, the N_2O emission increments and copy number of denitrifiers genes diminished over time. This study revealed divergent changes in soil nitrogen processes and highlighted N_2O emissions with a greater denitrification rate under microplastic exposure. The negative impacts of microplastics on soil health were revealed from the perspective of soil nitrogen availability and N_2O emissions.

1. Introduction

Currently, microplastics seriously endanger the health of ecosystems on a global scale (Allen et al., 2022; Rillig and Lehmann, 2020). Microplastics have been observed to not only negatively alter the biological activities and behaviors of soil (Zhang et al., 2022b), but also their biogeochemical cycles (Ingraffia et al., 2022; Zhu et al., 2021). Soil nitrogen cycling is essential in terrestrial ecosystems since it plays critical roles in plant growth and environmental issues (e.g., global warming) (Lehnert et al., 2021). Although microplastics contain negligible nitrogen, they can modify nitrogen cycling to a remarkable degree (Yu et al., 2021). Recently, much attention has been focused on the issue that microplastics can change soil nitrogen cycling (Gao et al., 2022; Li et al., 2022b; Shen et al., 2021; Zhang et al., 2022a,c). The previous studies began to focus on a few nitrogen formations (e.g., total soil nitrogen) (Gao et al., 2022; Li et al., 2022b; Zhang et al., 2022a,c), and an earlier study implied that several nitrogen formations were impacted by microplastics (Zhang et al., 2022a,c). More importantly, although a recent study found that the emission of nitrogen dioxide (N_2O) with a strong global warming potential was also changed under the exposure of microplastic (Zhang et al., 2022c), the mechanism of changed N_2O emission is still unclear. Further, the changes in soil nitrogen processes and the involved functional microorganisms have not been systematically elucidated at exposure to microplastics. Thus, it is urgent to ascertain how soil nitrogen processes and their typical products (e.g., N_2O) are changed under microplastics exposure.

Different forms of soil nitrogen (e.g., organic nitrogen, ammonium, nitrate, and nitrite) may be modified by microplastics; however, there is no consensus on the specific changes involved under microplastics exposure. The soil ammonium (NH_4^+) concentration was increased and

nitrate (NO_3^-) was decreased following the application of microplastics (Shi et al., 2022). Conversely, in another study, the concentrations of NH_4^+ and NO_3^- were not significantly affected (Jiao et al., 2022); thus, changes in soil nitrogen formations under microplastic exposure remain unclear. Soil processes convert nitrogen from one form to another one, which is helpful toward understanding the kinetics involved. Curiously, the alterations in soil nitrogen processes have not been synthetically emulated for study. Microplastics can change the community structures of soil microorganisms to a certain extent (Li et al., 2022a; Meng et al., 2023; You et al., 2022), and the alterations in functional microorganisms involved in nitrogen cycling may ultimately impact the corresponding nitrogen process (Rong et al., 2021). For example, ammonium-oxidizing microorganisms (e.g., *amoA* marker gene) mediate the initial step of the nitrification process, while functional microorganisms (e.g., marker genes including *narG*, *napA*, and *nirS*) play essential roles in the denitrification process (Qian et al., 2018; Song and Niu, 2022). An experimental study revealed that changes in the copy numbers of *amoA* and *nirS* induced by microplastics could closely reflect the dynamics of NH_4^+ and NO_3^- concentrations (Sun et al., 2021). Simultaneously, previous meta-analyses have not tested the changes of functional microorganisms involved in the nitrogen processes (Gao et al., 2022; Li et al., 2022b; Zhang et al., 2022a,c). For this study, we proposed a hypothesized framework to decipher the changes in soil nitrogen cycling under microplastic exposure, including nitrogen processes and functional microbes (Fig. 1). Additionally, functional genes are translated to enzymes that catalyze nitrogen processes. For example, nitrate reductase is encoded by *narG* or *napA* (Butala and Falkinham, 2018), whereas nitrite reductase is encoded by *nirS* and *nirK* (Graf et al., 2014), with both enzymes participating in denitrification (Shen et al., 2021). Shi et al., 2022 reported that under microplastics exposure,



Fig. 1. A proposed hypothesized framework to reveal the changes in nitrogen transformations responding to microplastics. The pink arrow shows the relationship between two nitrogen formations. The dashed lines indicate the hierarchical changes in the nitrogen cycling process. Functional microorganisms are in the pink rectangle, and soil enzymes are in the green rectangle. '?' Represents missing variable. Abbreviations: NR = Nitrate reductase, NIR = Nitrite reductase, NXR = Nitrite oxidoreductase.

decreasing soil nitrite (NO_2) concentrations were closely associated with higher nitrate reductase activities. Therefore, the enzyme activities involved in nitrogen cycling are indispensable variables for elucidating changes in nitrogen processes (Fig. 1).

The responses of soil N_2O emissions to microplastics remains controversial, however, as some researchers have reported that soil N_2O emissions were not impacted under microplastics exposure (Gao et al., 2020), while others speculated that they did induce N_2O emissions (Han et al., 2021). Although soil N_2O emissions may be altered following the addition of microplastics, the exact source of N_2O (i.e., nitrification or denitrification) has not been explored in previous meta-analyses (Fig. 1). Functional genes that govern N_2O emissions during nitrification and denitrification may respond variably to microplastics, as it has been reported that microplastics can alter the properties of soil (i.e., pH and large aggregate content, etc.) and impact soil aeration (Lu et al., 2021; Machado et al., 2017; Shi et al., 2022). The copy numbers of *nirS* were observed to either increase (Yu et al., 2022) or decrease under microplastics exposure (Seeley et al., 2020), while the magnitude of *amoA* was either not affected (Yu et al., 2022) or decreased (Seeley et al., 2020). The variable responses of these genes likely entangle the contributions of nitrification and denitrification to N_2O emissions (Fig. 1). Furthermore, it has been assumed that the effects of microplastics on soil nitrogen cycling were related to their characteristics (i.e., structure, particle size, concentration, etc.) and experimental duration (Gao et al., 2020; Inubushi et al., 2022). How these factors influence the responses of attributes in soil nitrogen cycling is needed to be revealed.

To systematically assess the impacts of microplastics on soil nitrogen cycling, we collected data from experimental studies to conduct a meta-analysis on a global scale. The core objectives of this study were to: (1) quantify the extent to which various soil nitrogen forms changed (particularly for the N_2O emission rate) under microplastic exposure; (2) reveal how soil nitrogen processes were altered and identify which dominated the modification of N_2O emissions; (3) explore how the properties of microplastics and experimental conditions influenced N_2O emissions and the functional microorganisms that induced them. This study deepened our understanding of the ecological risks of microplastics from the perspective of soil nitrogen cycling.

2. Materials and methods

2.1. Data collections

We examined peer-reviewed papers that related to the effects of microplastics on soil nitrogen cycling as of July 19, 2022, using the Web of Science (<http://apps.webofknowledge.com>) and China National Knowledge Infrastructure Database (<http://www.cnki.net>). The keyword strings employed to search for papers included: (('microplastic*' OR 'microbeads' OR 'microfiber' OR 'plastic debris' OR 'plastic fragment') AND (' N_2O ' OR 'nitrous oxide' OR 'nitrogen' OR 'enzyme*' OR 'pH' OR 'macroaggregate' OR 'MBC' OR 'microbial biomass carbon' OR 'DOC' OR 'dissolved organic carbon' OR 'nitrification rate' OR 'denitrification rate' OR 'nitrogen immobilization rate' OR 'nitrogen mineralization rate' OR '*amoA*' OR '*nxrA*' OR '*nxrB*' OR '*napA*' OR '*napB*' OR '*narG*' OR '*narB*' OR '*narH*' OR '*narI*' OR '*nasA*' OR '*nirK*' OR '*nirS*' OR '*norB*' OR '*nosZ*') AND ('soil' OR 'ecosystem*')). These keywords were chosen to cover the maximum number of published studies. The total numbers of selected papers from the Web of Science and China National Knowledge Infrastructure Database were 1171 and 197, respectively. Four criteria were used to identify eligible papers as follows: (1) the article that at least one variable pertaining soil nitrogen process was reported (e.g., soil N_2O emission, nitrogen components, soil enzymes, nitrogen process rates, functional genes, soil physical and chemical properties, etc.) was eligible; (2) the experiment was carried out with a strict control treatment (i.e., 'no microplastic' treatment) in the laboratory or in the field; (3) data from the microplastic and control treatments were pairwise collected when there were multiple treatments; (4)

target variables (e.g., denitrification rate) should report the mean, sample size, and standard deviation or standard error. In summary, 60 papers matched these criteria, of which 54 papers were from Web of Science and 6 papers were from China National Knowledge Infrastructure Database; thus, they were employed to develop the dataset for the meta-analysis. The PRISMA flow graph for constructing the dataset is shown in Fig. S1. The full list of papers is provided in the supplementary materials.

We collected the following variables: (1) soil N_2O emission rate; (2) concentrations of soil nitrogen forms: ammonium, nitrate, nitrite, total nitrogen, dissolved nitrogen, dissolved organic nitrogen, and microbial biomass nitrogen; (3) activities of soil enzymes: nitrate reductase, leucine aminopeptidase, hydroxylamine reductase, urease, chitinase, protease, and polyphenol oxidase; (4) nitrogen process rates: nitrification, denitrification, mineralization, and immobilization rates; (5) copies of functional genes over nitrogen processes: *amoA*, *nxrA*, *nxrB*, *napA*, *napB*, *narG*, *narB*, *narH*, *narI*, *nasA*, *nirK*, *nirS*, *norB*, and *nosZ*; (6) soil physical and chemical properties: pH, microbial biomass carbon, percentage of macroaggregates, and dissolved organic carbon concentration; (7) microplastic properties: shape, particle size, and concentration; (8) experimental conditions: experimental duration.

Further, we extracted experimental details, such as the number of replicates for each treatment. Data were taken from tables or figures in the main text or supplementary materials. We extracted data using GetData graph digitizer software (<http://getdata-graph-digitizer.com/>), while the data were shown as images. If the paper provided only the median it was converted to the mean according to Luo et al. (2015). If only the standard error (SE) was provided in an article, the SE was converted to SD using the formula $SD = SE * \sqrt{n}$. For studies without SD or SE, SD was calculated as 1/10 of the mean (Su et al., 2022).

2.2. Data analyses

The effects of microplastics on individual variables were quantified by calculating the natural logarithm of the response ratio ($\ln RR$) in this study. We chose to use $\ln RR$ for this meta-analysis, as the calculation is not biased in the face of discrepant sample sizes between treatments, and the data typically follows a normal distribution (Li et al., 2020; Vidal and Murphy, 2018; Hedges et al., 1999). The calculation equation is:

$$\ln RR = \ln\left(\frac{\bar{X}_T}{\bar{X}_C}\right) = \ln(\bar{X}_T) - \ln(\bar{X}_C) \quad (1)$$

where \bar{X}_T and \bar{X}_C denote the mean values of the variable under the microplastic treatment and control treatment, respectively, and the variance (v) of $\ln RR$ was calculated as follows:

$$v = \frac{S_T^2}{n_T \bar{X}_T} + \frac{S_C^2}{n_C \bar{X}_C} \quad (2)$$

where S_T and S_C are the standard deviations of the microplastic and control treatments, respectively; n_T and n_C denote the number of experimental replicates of the microplastic and control treatments, respectively.

We used the random effects model to calculate the $\ln RR$ and its variance, since the random effects model takes into account the variation of effect values between sites (Kpemoua et al., 2022; Ferlian et al., 2018; McKenzie et al., 2016). In random-effect models, there are two sources of variances, including the within-study variance (v_i) and between-study variance (τ^2). The weight factor w_i was calculated using:

$$w_i = \frac{1}{v_i + \tau^2} \quad (3)$$

$$RR_{++} = \frac{\sum_{i=1}^m w_i \ln RR}{\sum_{i=1}^m w_i} \quad (4)$$

where i is the number of observations.

We estimated the mean effect size and 95 % confidence interval (95% CI) for each variable.

$$95\%CI = RR_{++} \pm 1.96SE(RR_{++}) \quad (5)$$

where $SE(RR_{++})$ is the standard error of the weighted response ratio (RR_{++}).

$$SE(RR_{++}) = \sqrt{\frac{1}{\sum_{i=1}^m w_i}} \quad (6)$$

If the 95% CI of the variable's effect size overlapped '0', it indicated that this variable was negligibly affected by microplastics; otherwise, the variable was significantly altered by the microplastics.

The percentage change of a variable was obtained by

$$(e^{RR_{++}} - 1) \times 100\% \quad (7)$$

We also separately compared the effect values of microplastics on the soil N_2O concentrations and functional genes in terms of nitrogen cycling between different groups. For instance, the dataset was divided into four groups according to microplastic shapes: fibrous, fragmented, round, and irregular; two groups based on the microplastic particle size: < 1 mm and > 1 mm; three groups according to microplastic concentration ranges: < 10 g/kg, 10–50 g/kg, and > 50 g/kg; and four groups based on experimental durations: < 5 days, 5–15 days, 15–30 days, and > 30 days. The effect size of each variable was calculated by using OpenMEE software (<http://www.cebm.brown.edu/openmee/index.html>).

Finally, we tested the conceptual framework (Fig. 5) to elucidate the kinetics behind changes in soil nitrogen cycling in response to microplastic exposure. In particular, to show how N_2O emissions and the concentrations of nitrate, nitrite, and ammonium changed with nitrogen processes on exposure to microplastics. We used linear mixed-effect models to obtain the p values and coefficient values between variables. In each mixed-effects model, the fixed-effect is the response of each variable to the microplastic, while the random-effect takes into account the difference in the response of each variable to the microplastic between different cases.

3. Results

3.1. Changes in soil nitrogen formations and N_2O emissions under microplastics exposure

The contents of a series of soil nitrogen formations and the overall N_2O emission rate were influenced by microplastics (Fig. 2). The contents of ammonium, nitrate, and total soil nitrogen were reduced by 6.7 % (95 %CI: –13.2 % to –0.1 %), 22.4% (95 %CI: –30.1 % to –14.7 %), and 4.3% (95 %CI: –8.0 % to –0.6 %), respectively, under microplastics exposure. In contrast, the concentrations of nitrite, dissolved nitrogen, and microbial biomass nitrogen were increased by 38.8 % (95 %CI: 10.5–67.1 %), 8.6 % (95 %CI: 2.1–15.1 %), and 27.4 % (95 %CI: 5.1–49.6%), respectively. More importantly, soil N_2O emission rate was significantly enhanced by microplastics (140.6 %; 95 %CI: 102.1–179.1 %).

3.2. Changes in soil enzymatic activities and nitrogen processes induced by microplastics

The activities of soil enzymes were significantly stimulated under microplastic exposure (Fig. 3a). The activities of soil resident nitrate reductase, leucine aminopeptidase, urease, and chitinase were increased by 4.8 % (95 %CI: 0.4–9.2 %), 17.5 % (95 %CI: 8.9–26.0 %), 6.9 % (95 %CI: 2.7–11.1 %), and 6.7 % (95 %CI: 0.1–13.3 %), respectively. Conversely, hydroxylamine reductase and polyphenol oxidase were not significantly affected. The denitrification rate (17.8 %; 95 %CI: 1.5–34.1 %) was stimulated by microplastics, while the nitrification rate, nitrogen



Fig. 2. Effects of microplastics on soil nitrogen formations and N_2O emissions. Error bars represent 95% confidence intervals. The response ratio is significantly different from zero if the 95% CI of the effect size does not overlap zero. The number around the error bars is the sample size of each variable. *, $p < 0.05$, **, $p < 0.005$, and ***, $p < 0.001$, respectively. Abbreviations: NH_4^+ = ammonium, NO_3^- = nitrate, NO_2^- = nitrite, TN = total nitrogen, DN = dissolved nitrogen, and MBN = microbial biomass nitrogen.



Fig. 3. Changes in soil enzymatic activities (a) and the rates of nitrogen processes in response to microplastics (b). Error bars represent 95% confidence intervals. The response ratio is significantly different from zero if the 95% CI of the effect size does not overlap zero. The number around the error bars is the sample size of each variable. *, $p < 0.05$, **, $p < 0.005$, and ***, $p < 0.001$, respectively. Abbreviations: NR = Nitrate reductase, LAP = Leucine aminopeptidase, HR = Hydroxylamine reductase, UE = Urease, PT = Protease, PPO = Polyphenol oxidase, Nit rate = Nitrification rate, Den rate = Denitrification rate, N_1 rate = Nitrogen immobilization rate, N_{min} rate = Nitrogen mineralization rate.

immobilization rate, and nitrogen mineralization rate were all likely increased by microplastics in spite of negligible changes (Fig. 3b).

3.3. Changes in the copy numbers of functional genes for both soil nitrification and denitrification under microplastics exposure

The total copies of functional genes associated with nitrification processes were not significantly altered by microplastics (Fig. 4a). Specifically, copies of both *amo* and *nxr* were not significantly affected by microplastics. Unlike nitrification, the total copy numbers of functional genes involved in denitrification were stimulated by microplastics (10.6 %; 95 %CI: 0.1–21.1 %) (Fig. 4b). Specifically, during the reduction of NO_3^- to NO_2^- , the copy numbers of *nap* (115.3 %; 95 %CI: 86.8–143.9 %) and *nas* (23.1 %; 95 %CI: 19.9–26.4 %) were significantly increased, while the copy number of *nar* (–4.8 %; 95 %CI: –8.5 % to –1.1%) was slightly inhibited under microplastics exposure. Microplastics also increased the copy numbers of *nir* by 18.4 % (95 %CI:

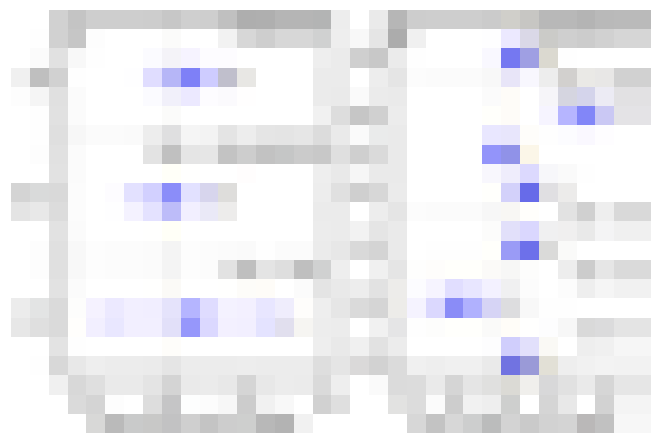


Fig. 4. Changes in soil functional genes over nitrification (a) and denitrification (b) under microplastics exposure. Error bars represent 95% confidence intervals. The response ratio is significantly different from zero if the 95% CI of the effect size does not overlap zero. The number around the error bars is the sample size of each variable. *, $p < 0.05$, **, $p < 0.005$, and ***, $p < 0.001$, respectively. The amo includes amoA, amoB, and amoC; nxr is nxrA; nap includes napA and napB; nar includes narB, narG, narH, and narI; nas is nasA; nir includes nirK and nirS; nor includes norB and norC; and nos is nosZ.

4.8–32.1 %) during NO_2 reduction to N_2O and increased the *nos* copy numbers that governed N_2O reduction by 11.0 % (95 %CI: 3.1–18.9 %). The increments of *nir* copy numbers were greater than that of *nos* copy numbers under microplastics exposure. A negative response for the copy number of *nor* was observed under microplastic exposure (–36.7 %; 95 %CI: –66.6 % to –6.9 %).

3.4. Underlying mechanisms of changes in soil nitrogen cycling under microplastics exposure

There are two main pathways through which microplastics can affect soil N_2O emissions. On the one hand, microplastics can impact the

turnover of soil nitrogen and subsequent N_2O emissions (Fig. 5). Specifically, microplastics increased the abundance of functional denitrifiers, leading to an increase in the denitrifying rate. In particular, the rate of nitrate reduction (transformation of NO_3^- to NO_2^-) was enhanced due to additional copies of *nap* and *nas*, as was the rate of nitrite reduction deduced from the additional copies of *nir*. The changes in these two processes led to decreased NO_3^- concentrations (0.66, $p = 0.00$) and increased NO_2^- during the denitrification process, which eventually increased the N_2O emissions. There was no significant relationship between NH_4^+ and NO_2^- , except for the contribution of nitrification to N_2O emissions. Furthermore, the reduction of the nitrification rate was responsible for lower NO_3^- concentrations, since the concentration of NO_3^- was positively correlated with the NH_4^+ concentrations (0.17, $p = 0.04$).

Conversely, microplastics influenced N_2O emissions by transforming the physicochemical properties of the soil (Fig. 5), as the number of soil macroaggregates was increased. Dissolved organic carbon in the soil was also boosted due to microplastics exposure. Consequently, due to the greater number of macroaggregates and higher level of dissolved organic carbon (0.42, $p = 0.03$) the soil microbial biomass carbon was increased. Additionally, a higher soil pH was responsible for the increment of soil microbial biomass carbon (0.64, $p = 0.02$). These changes in soil attributes induced modifications in the soil nitrogen cycling. Although the higher soil pH suppressed soil N_2O emissions to some extent (–0.50, $p = 0.00$), the high level of microbial biomass carbon stimulated N_2O production to a greater degree (0.75, $p = 0.03$). Considering the negative relationship between NO_3^- concentrations and the microbial biomass carbon (0.90, $p = 0.04$) in conjunction with the positive relationship between the N_2O emission rate and microbial biomass carbon (0.75, $p = 0.03$), it was suggested that additional microbial biomass carbon promoted denitrification and ultimately increased N_2O emissions.

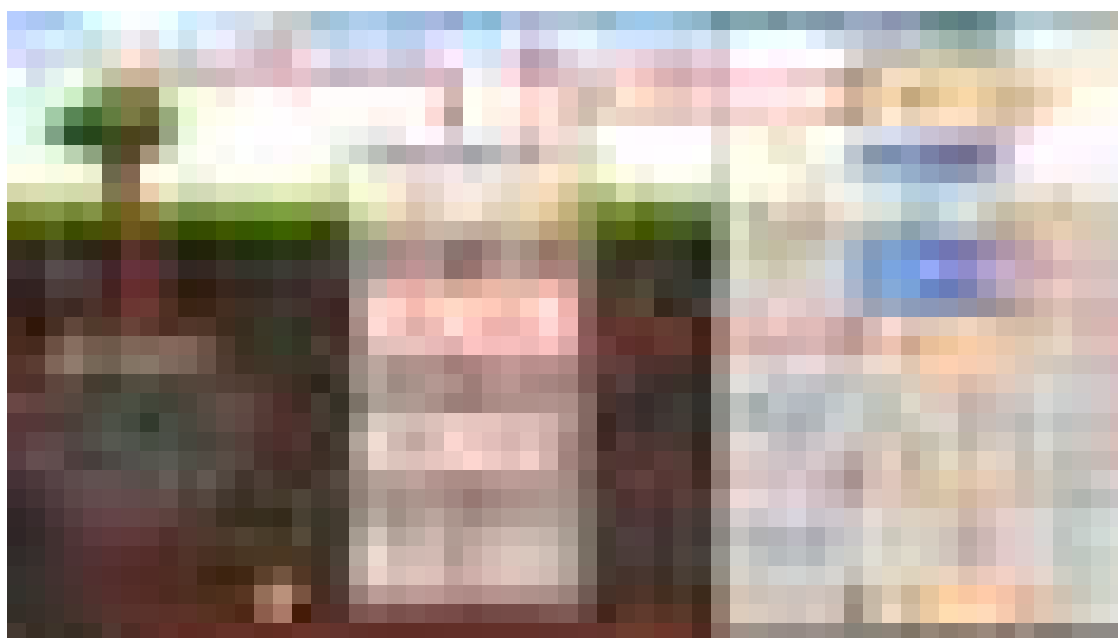


Fig. 5. Conceptual diagram of potential mechanisms of changes in soil nitrogen cycling under microplastic exposure. ↓ denotes a significant decrease, ↑ denotes a significant increase, – denotes no significant change. The + denotes a positive correlation. The red solid lines indicate that the correlation is significant, and the red dashed lines indicate that the correlation is not significant. The black dashed line indicates an unproven relationship. Figures on the lines are correlation coefficients that negative value indicates a negative effect and positive value indicates a positive effect. *, $p < 0.05$, **, $p < 0.005$, and ***, $p < 0.001$, respectively. MB, SOM and DON are microbial biomass, soil organic matter, and dissolved organic nitrogen, respectively.

3.5. Changes in N₂O emissions and functional genes during denitrification induced by different microplastic attributes

Among their various morphologies, round and irregularly shaped microplastics increased the soil N₂O emissions by 114.5% (95 %CI: 106.2–122.7 %) and 85.9 % (95 %CI: 44.3–127.5 %), respectively. In contrast, fibrous microplastics did not significantly impact N₂O emissions ($p = 0.96$) (Fig. 6a). In terms of microplastic dimensions, the N₂O emissions were increased by 85.9 % (95 %CI: 44.3–127.5 %) in response to particle sizes of < 1 mm, and by 165.9 % (95 %CI: 108.6–223.2 %) in response to particle sizes of > 1 mm (Fig. 6a). As relates to microplastic concentrations, the N₂O emissions were increased by 171.8 % (95 %CI: 82.6–261.1 %) at < 10 g/kg; 105.6 % (95%CI: 83.6–127.7 %) at 10–50 g/kg; and 96.0% (95 %CI: 79.6–112.4 %) at > 50 g/kg (Fig. 6b). There were no significant differences in the response ratios of N₂O emissions between these three categories. Regardless of experimental duration, the soil N₂O emissions were consistently higher under microplastics exposure (Fig. 6b). Interestingly, higher levels of soil N₂O emissions were observed under microplastics exposure with an experimental duration of less than 15 days, than those over longer durations (15–30 days and > 30 days).

In terms of gene copy numbers, fragmented (18.1 %; 95 %CI: 1.8–34.4 %), round (11.3 %; 95 %CI: 2.5–20.1 %), and irregularly shaped (18.2 %; 95 %CI: 1.9–34.5 %) microplastics upregulated the copy numbers of functional genes during denitrification (Fig. 7). The gene copy numbers under microplastics exposure during denitrification always increased, regardless of microplastic concentrations. Moreover, this response ratio decreased with higher microplastic concentration gradients. When the experimental duration was < 15 days, the copy numbers of functional genes during denitrification increased by 109.8 % (95 %CI: 72.0–147.7 %), which was much greater than the response ratio at longer exposure times (e.g., >30 days; 7.4 %; 95 %CI: 0.7–14.0 %).

4. Discussion

4.1. Increases in N₂O under microplastic exposure was mainly due to denitrification

Soil nitrogen cycling was observed to be profoundly affected by microplastics, and more importantly, soil N₂O emissions were dramatically increased (Fig. 2). Shen et al. (2021) summarized the recent advances in the impacts of microplastics on nitrogen cycling, but not conduct quantitative analysis. You et al. (2022) used bibliometric

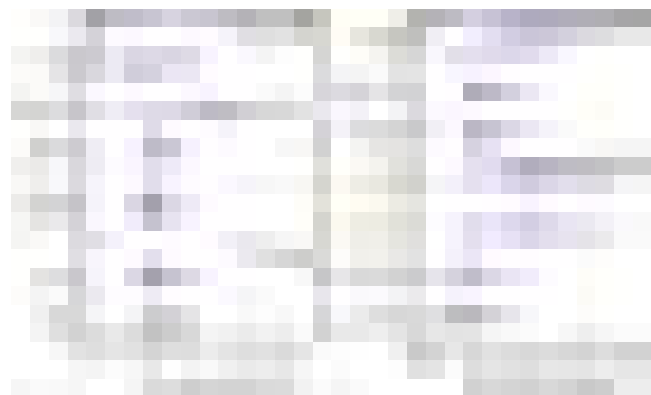


Fig. 6. Effects of microplastic properties and experimental duration on N₂O emission. Error bars represent 95% confidence intervals. The response ratio is significantly different from zero if the 95% CI of the effect size does not overlap zero. The number around the error bars is the sample size of each variable. *, $p < 0.05$, **, $p < 0.005$, and ***, $p < 0.001$, respectively. Abbreviations: MPs = microplastics.



Fig. 7. Effects of microplastic properties and experimental duration on denitrification functional genes. Error bars represent 95% confidence intervals. The response ratio is significantly different from zero if the 95% CI of the effect size does not overlap zero. The number around the error bars is the sample size of each variable. *, $p < 0.05$, **, $p < 0.005$, and ***, $p < 0.001$, respectively. Abbreviations: MPs = microplastics.

methods to review the abundance, distribution, and interaction of microplastics in soil ecosystems, but not study the nitrogen cycle. In recent studies, researchers began to use meta-analysis to only focus on microplastics induced changes on soil physicochemical properties and a few nitrogen forms (Li et al., 2022b). Zhang et al., 2022a,c revealed that the nitrate content was increased by 12 %, while ammonium and nitrite were not significantly affected by microplastics based on a small dataset. By employing an extensive dataset, according to the transformations in soil nitrogen processes, we demonstrated that not only the total soil nitrogen and nitrate, but also ammonium and nitrite concentrations were significantly impacted under microplastics exposure. Changes in the total soil nitrogen, ammonium, and nitrate can influence soil N₂O emissions (Li et al., 2022c). This study revealed that N₂O emissions increased in nitrogen cycling (Figs. 2, 5), albeit that the N₂O emissions may be reduced under microplastics exposure using a small data set (Zhang et al., 2022c). This may be related to fertilization and plant growth (Gao et al., 2020; Ren et al., 2019; Rillig et al., 2021). For instance, Ren et al. (2019) found that microplastics reduced soil N₂O emissions in fertilized soils. However, Cheng et al. (2022) found that microplastics increased N₂O emissions by up to 69.3 % in sediments, which was similar to this study. Therefore, we speculated that microplastics induced a stronger greenhouse gas potential in soil ecosystems due to the substantial increase in N₂O emissions.

The increases in N₂O were primarily derived from the increments of the denitrification rate under microplastics exposure, since the contribution of denitrification to N₂O emission was stronger than that of nitrification (Figs. 2, 3b, 4). Since recent meta-analyses did not test the changes in functional microorganisms during nitrogen cycling, and the corresponding processes under exposure to microplastics, it was difficult to elucidate the origin of N₂O. This study found that microplastics not only induced a significant increase in the population of denitrifiers, but also led to a simultaneous increase in the denitrification rate (Figs. 3b, 4b). Notably, both the copy numbers of nitrifying microbes and the nitrification rate were not significantly altered under microplastics exposure (Figs. 3b, 4a). Su et al., 2022 and Cheng et al. (2022) recently identified the N₂O production pathway in estuarine sediments by isotope labeling. It was revealed that higher N₂O generation was the result of bacterial and fungal denitrification rather than chemodenitrification. Thus, substantial soil N₂O emissions may be also generated through the biotic denitrification pathway.

Why do microplastics increase denitrification and N₂O emissions? It was reported that microplastic surfaces are attractive to microorganisms, which can form a “microplastic sphere” on the surfaces of microplastics following original colonization, growth, and maturation (Sun

et al., 2022). Recently, Su et al., 2022 and Cheng et al. (2022) pointed out that these microplastic spheres were more likely to recruit denitrifiers for colonization. This was mainly due to the highly hypoxic conditions within the microplastic spheres, where denitrifiers utilized nitrate/nitrite as electron acceptors rather than O_2 to sustain their metabolism (Yang et al., 2022). The microplastics did indeed provide an ecological niche for several denitrifying bacteria (Cheng et al., 2022). For example, the abundance of *Bacillus*, *Pseudomonas*, *Paracoccus*, and *Acinetobacter* were observed to increase. Additionally, the carbon content of microplastics is relatively high, and some denitrifiers were apt to live in the condition with enriched carbon (Ding et al., 2022). Thus, the anoxic habitats of the “microplastic spheres” can lead to higher denitrification rates. During the denitrification process, the copy numbers of *nap*, *nas*, and *nir* were increased on exposure to microplastics, and the ratios of both *nap/nir* and *nas/nir* were greater than 1 (Fig. 5). This suggested that nitrate reduction was enhanced to provide more substrates for nitrite reduction, which consequently increased N_2O emissions. Our speculation aligned with the findings of Rong et al. (2021), who reported that microplastics increased the copy number of the functional gene that encoded for nitrite reductase (*nirK*) and N_2O production through denitrification in upland soils.

Microplastics can also indirectly influence denitrification processes by altering physicochemical soil properties. Notably, increased microbial soil biomass stimulated the denitrification rate (Fig. 5). Greater microbial biomass may support more highly active microorganisms to participate in denitrification to generate N_2O under anoxic conditions (Patoine et al., 2022). Further, although Li et al., 2022d found a positive correlation between the denitrification rate and pH, we found that low pH was more conducive for denitrification to produce N_2O (Fig. 5). This may have been due to the low pH, which limited N_2O reduction and resulted in a higher proportion of $N_2O:N_2$ during denitrification; thus, more N_2O was emitted (Liao et al., 2021).

4.2. Effects of microplastics on the transformation of soil nitrogen

Nitrogen processes were modified to different degrees, which resulted in variable changes in soil nitrogen forms under microplastics exposure. Between nitrogen immobilization and mineralization, microplastics increased the activities of urease (Fig. 3) through which a portion of organic nitrogen was catalyzed to NH_4^+ (Fei et al., 2019). However, microplastics did not significantly increase the nitrogen immobilization rate. Interestingly, the concentrations of NH_4^+ were reduced, which may have been due to microplastics increasing the abundance of several microbes (e.g., *Thiobacillus*), which can consume NH_4^+ (Sun et al., 2021; Yu et al., 2015). The decrease in NH_4^+ limited the growth of nitrifiers (e.g., *amo*) somewhat, as well as the nitrification rate (Figs. 3a, 4a), which was consistent with the findings of Gao et al. (2020). The *amo* is responsible for ammonification, which is the initial rate-limiting step of nitrification (Wang et al., 2022), which when constrained, leads to a lower NO_3^- concentration to some extent. In contrast, during denitrification, *nas* and *nap*, which are responsible for assimilating and dissimilating nitrate reduction, were increased by microplastics. The corresponding enzymes of these two functional genes converted NO_3^- to NO_2^- (Nelson et al., 2016), and the activities of nitrate reductase were increased (Fig. 3a). As a result, the concentration of NO_3^- reduced and the nitrite concentration increased. Collectively, microplastics impacted the relative abundances of functional genes involved in nitrogen cycling and the activities of soil enzymes, which brought about various changes in nitrogen forms.

Fluctuations in nitrogen transformations were also induced by changes in the microbial biomass under microplastics exposure. As is known, soil microbial biomass is crucial for nitrogen cycling and nitrogen availability (Li et al., 2019). The increases in microbial biomass on exposure to microplastic may have been due to the following reasons. Firstly, soil macroaggregates were induced to form under microplastic exposure, which may be suitable for microbial growth. A field

investigation showed that plastic particulates were found in 72 % of soil aggregates (Wang et al., 2022), which was primarily due to the microplastics participating in soil aggregation together with plant roots and soil particles (Zhang, 2018). Microplastics or their intermediates can be transformed to soil soluble carbon by bacteria (Wang et al., 2021); thus, with better soil structure and more available carbon, the microbial biomass carbon increased (Fig. 5). Secondly, high soil pH has a strong impact on the dynamics of soil microbes. Changes to bacterial communities by microplastics in alkaline soils are greater than those in acidic soils (Liu et al., 2020). Moreover, higher pH is conducive to greater microbial biomass (Malik et al., 2018), which regulated nitrogen processes and resultant nitrogen forms (Fig. 5).

4.3. Time-dependent soil N_2O emissions under microplastics exposure

The N_2O emissions under microplastic exposure were much higher over the short term (Fig. 6b). Similarly, Ren et al. (2019) reported a 20.3 % increase in N_2O emissions on the 3rd day after microplastics addition, but only a 1.3 % increase on the 15th day. The changes in functional microorganisms were also time dependent. We found that the increment in the copy numbers of functional genes during denitrification decreased over time (Fig. 7). Consequently, N_2O production during denitrification may exhibit a surge over the short term. The abundance of *Nitrospirae* was observed to decrease following long-term exposure to microplastics (Sun et al., 2021). Shortages in denitrifying substrates may limit increases in N_2O , as *Nitrospirae* can transform NO_2^- to NO_3^- (Wang et al., 2018). Furthermore, the stimulating effect of microplastics on the bioavailability of nitrogen is temporary. Microorganisms prefer to consume soluble substances (i.e., inorganic nitrogen and total dissolved nitrogen) to meet their metabolic demands, which includes increasing their biomass or enlarging their population (Rillig et al., 2021; Yuan et al., 2018). Microplastics cannot provide sufficient nutrients for the metabolic activities of microorganisms, as they contain little nitrogen.

4.4. Limitations

This meta-analysis revealed that microplastics primarily altered the denitrification process of the nitrogen cycle, which stimulated N_2O emissions. However, this study had some limitations. Firstly, most observations regarding the effects of microplastics on the transformation of soil nitrogen and N_2O emission were performed within one year; thus, there was a lack of long-term experimental data. In our study, the longest experimental duration was 104 days, with an average of only 21 days. Although microplastics were shown to increase N_2O emissions, the results from short-term experiments were not able to predict the consequences over the long run (Yu et al., 2022), as we found that the increments of N_2O and functional genes diminished over time. Accordingly, long-term experiments under microplastic exposure are of considerable interest toward estimating the future impacts of microplastics pollution on nitrogen cycling. In addition, in future studies, we should also use molecular biology and stable isotope techniques to further explore the mechanisms for the effect of microplastics on soil health.

Secondly, we did not investigate the influences of the shapes, sizes, and concentrations of microplastics on N_2O emissions. A previous study reported that smaller microplastics were more likely to block soil micropores and alter soil aeration (Xiang et al., 2022). Lehmann et al. (2021) confirmed through their experiments that microplastics had a shape-dependent effect on soil aggregation and organic matter loss. Furthermore, the activities of certain microorganisms and enzymes involved in the nitrogen cycle were inhibited under high microplastic concentrations (Fan et al., 2022; Yi et al., 2020). Therefore, the impacts of microplastics on soil N_2O emissions may vary for different microplastics, and the underlying mechanisms remain to be explored.

Thirdly, although we revealed that denitrification dominated the generation of higher N_2O emissions from the perspective of soil

physicochemical properties, Guo et al. (2022) proposed that “soil-microplastic mixed particles” would affect the soil water content. The soil water content was considered to be an essential controlling factor for soil N₂O emissions, as it may regulate microbial activities during nitrogen cycling (Zhang et al., 2021). The dynamics of the soil water content were not tested due to data limitations. It is indispensable to incorporate additional key soil physicochemical factors to decipher the underlying kinetics of increased N₂O emissions and denitrification rates under microplastics exposure.

5. Conclusions

In contrast to earlier studies, this investigation systematically quantified the effects of microplastics on soil nitrogen forms, nitrogen processes, and N₂O emissions, and revealed the sources of increased N₂O emissions under microplastics exposure. Soil N₂O emissions eventually increased by 140.6% under microplastics exposure. The denitrification rate was significantly increased by 17.8% on exposure to microplastics. It is worth noting that the contribution of denitrification to N₂O increments was greater than that of nitrification. The total number of denitrifiers genes was increased by 10.6%, as were the copy numbers of several genes (*nap*, *nas*, *nir*, and *nos*); however, the copy numbers of nitrifiers were not altered under microplastics exposure. Furthermore, microplastics significantly increased the activities of nitrate reductase by 4.8%. The divergent changes in soil nitrogen processes resulted in decreased soil ammonium and nitrate concentrations (−6.7% and −22.4%, respectively), and increased nitrite concentrations (38.8%). The increments of N₂O emissions and denitrifiers functional genes were likely to diminish over longer experimental durations. The divergent changes in different soil processes suggested that the processes in soil nitrogen turnover were heterogeneously altered by microplastics. Higher N₂O emissions, as well as lower ammonium and nitrate concentrations may be harmful to soil health under microplastics exposure.

CRedit authorship contribution statement

Pinjie Su: Conceptualization, Methodology, Data Collection, Visualization, Formal analysis, Writing – original draft. **Changyuan Gao:** Conceptualization, Methodology, Data Collection, Visualization, Formal analysis, Writing – review & editing. **Xiaojing Zhang:** Data Collection, Visualization. **Dan Zhang:** Methodology, Visualization. **Xingyu Liu:** Methodology, Formal analysis. **Tingting Xiang:** Data Collection, Visualization. **Yifu Luo:** Formal analysis, Writing – review & editing. **Kuo Chu:** Data Collection, Visualization. **Guohui Zhang:** Visualization, Writing – review & editing. **Naishun Bu:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Zhaolei Li:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing, 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.

Data Availability

Data will be made available on request.

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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.2022.130500.

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