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Stimulation of ammonia oxidizer and denitrifier abundances by nitrogen loading: Poor predictability for increased soil N_2O emission

Yong Zhang[1] | Feng Zhang[1] | Diego Abalos[2] | Yiqi Luo[3] | Dafeng Hui[4] | Bruce A. Hungate[3] | Pablo García-Palacios[5,6] | Yakov Kuzyakov[7,8,9] | Jørgen Eivind Olesen[2,10,11] | Uffe Jørgensen[2,11] | Ji Chen[2,10,11]

[1]School of Resources and Environmental Engineering, Anhui University, Hefei, China

[2]Department of Agroecology, Aarhus University, Tjele, Denmark

[3]Center for Ecosystem Science and Society and Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona, USA

[4]Department of Biological Sciences, Tennessee State University, Nashville, Tennessee, USA

[5]Departamento de Biología y Geología, Física y Química Inorgánica y Analítica, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, Móstoles, Spain

[6]Instituto de Ciencias Agrarias, Consejo Superior de Investigaciones Científicas, Madrid, Spain

[7]Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany

[8]Agro-Technological Institute, RUDN University, Moscow, Russia

[9]Institute of Environmental Sciences, Kazan Federal University, Kazan, Russia

[10]iCLIMATE Interdisciplinary Centre for Climate Change, Aarhus University, Roskilde, Denmark

[11]Aarhus University Centre for Circular Bioeconomy, Aarhus University, Tjele, Denmark

Correspondence
Feng Zhang, School of Resources and Environmental Engineering, Anhui University, Hefei 230601, China.
Email: fzhang188@163.com

Ji Chen, Department of Agroecology, Aarhus University, 8830 Tjele, Denmark.
Email: ji.chen.eco@gmail.com

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Abstract

Unprecedented nitrogen (N) inputs into terrestrial ecosystems have profoundly altered soil N cycling. Ammonia oxidizers and denitrifiers are the main producers of nitrous oxide (N_2O), but it remains unclear how ammonia oxidizer and denitrifier abundances will respond to N loading and whether their responses can predict N-induced changes in soil N_2O emission. By synthesizing 101 field studies worldwide, we showed that N loading significantly increased ammonia oxidizer abundance by 107% and denitrifier abundance by 45%. The increases in both ammonia oxidizer and denitrifier abundances were primarily explained by N loading form, and more specifically, organic N loading had stronger effects on their abundances than mineral N loading. Nitrogen loading increased soil N_2O emission by 261%, whereas there was no clear relationship between changes in soil N_2O emission and shifts in ammonia oxidizer and denitrifier abundances. Our field-based results challenge the laboratory-based hypothesis that increased ammonia oxidizer and denitrifier abundances by N loading would directly cause higher soil N_2O emission. Instead, key abiotic factors (mean annual precipitation, soil pH, soil C:N ratio, and ecosystem type) explained N-induced changes in soil

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N_2O emission. Altogether, these findings highlight the need for considering the roles of key abiotic factors in regulating soil N transformations under N loading to better understand the microbially mediated soil N_2O emission.

KEYWORDS
biological and chemical processes, denitrification, microbial gene abundance, nitrification, nitrogen addition, nitrous oxide, precipitation, soil pH

1 | INTRODUCTION

Terrestrial ecosystems continue to receive increasing nitrogen (N) inputs (Galloway et al., 2008; Kuypers et al., 2018). Some inputs are direct by fertilizer addition (Maaz et al., 2021), and others occur indirectly through atmospheric deposition (Yang et al., 2021). Excessive N loading has substantially disturbed ecosystem N-cycling processes, contributing to N losses (Huddell et al., 2020) and climate change (Sutton et al., 2011). For example, global nitrous oxide (N_2O) emission from N loadings has increased by more than 30% over the past four decades (Tian et al., 2020). Increased N_2O emission alters greenhouse gas balances, offsetting climate benefits from CO_2 removal and other climate solutions (Guenet et al., 2021; Liu & Greaver, 2009). The growth of both N_2O emission and its atmospheric burden underscores the urgency to effectively mitigate N-induced N_2O emission.

Ammonia oxidizers and denitrifiers are the main producers of N_2O (Stein, 2020), thus knowledge of how they respond to N loading may help develop mitigation strategies for soil N_2O emission (Wrage et al., 2004). Ouyang et al. (2018) found that N loading significantly increased ammonia oxidizer and denitrifier abundances in croplands, but the responses from other ecosystems and particularly their links with soil N_2O emission are still elusive (Hartmann et al., 2013; Tang et al., 2019; Zhang et al., 2013). Furthermore, the relative contribution of environmental and management factors in driving the responses of ammonia oxidizer and denitrifier abundances to N loading is unclear. In some studies N loading protocols (e.g. form and rate) primarily modulated the responses of ammonia oxidizer and denitrifier abundances (Fan et al., 2011; Luo et al., 2018), while other studies identified climate, vegetation and edaphic conditions as major drivers (Kong et al., 2010; Trivedi et al., 2019; Zhang et al., 2017). These knowledge gaps and uncertainties impede further understanding of microbial N transformations and soil N_2O emission under N loading.

Ammonia oxidizers dominate N_2O production in aerobic nitrification through the ammonia monooxygenase, which catalyzes the oxidation of ammonia (Prosser et al., 2020). In facultative anaerobic denitrification, denitrifiers drive the reduction of nitrate to N_2O by a series of enzymes (Philippot et al., 2007). Therefore, it has been hypothesized that soil N_2O emission is best explained by ammonia oxidizer and denitrifier abundances, and some studies even attempt to use the relationships between them to predict soil N_2O emission (Hu et al., 2015; Li et al., 2020; Linton et al., 2020; Morales et al., 2010; Ouyang et al., 2018). Despite the empirical support from several laboratory experiments for this hypothesis (Hink et al., 2018; Jones et al., 2014; Qiu et al., 2019), the relationships between soil N_2O emission and ammonia oxidizer and denitrifier abundances under field conditions are still a fertile arena of research debates. For example, some studies reported that abiotic factors rather than microbial abundance and microbial biomass were the key predictors of soil N_2O emission, as abiotic factors regulated a range of processes that related to soil N_2O emission, for example, nitrification and denitrification (Graham et al., 2014; Hartmann et al., 2013; Lammel et al., 2015; Pärn et al., 2018; Wang et al., 2018).

To address current challenges and test the laboratory-based hypothesis, we compiled a comprehensive global database of 101 field studies that manipulated N loading experiments in croplands, grasslands, and forests (Data S1–S4). For each study, we primarily recorded response variables including ammonia oxidizer abundance, denitrifier abundance, and soil N_2O emission. Meanwhile, a wide variety of environmental and experimental factors were documented as predictor variables. An advanced model selection analysis was combined with the conventional meta-analysis to investigate the responses of ammonia oxidizers, denitrifiers, and soil N_2O emission to N loading. Two questions motivated our study: (1) what are the key factors regulating the effects of N loading on ammonia oxidizer and denitrifier abundances, and (2) are there some links between the responses of ammonia oxidizer, denitrifier abundances to N loading and the responses of soil N_2O emission?

2 | MATERIALS AND METHODS

2.1 | Soil ammonia oxidizer and denitrifier abundances

Archaeal (*AOA*) and bacterial (*AOB*) *amoA* genes (encoding ammonia monooxygenase) and *nirK*/*nirS* genes (encoding nitrite reductase) are respectively used as functional markers of ammonia oxidizers and denitrifiers (Kuypers et al., 2018; Levy-Booth et al., 2014; Stein, 2020). The data availability for the *nosZ* gene (encoding N_2O reductase) is much more limited than for the other genes. Thus, we do not include *nosZ*-type denitrifier when referring to “denitrifiers” (although we collected the available data on *nosZ*-I gene and used it for exploratory analysis). The number of gene copies is the proxy for the abundance of the corresponding microbial guild. We searched relevant peer-reviewed articles published before 2021 using Web of Science (http://apps.webofknowledge.com/) and Google Scholar (https://scholar.google.com/). The keywords used were: (i) "nitrogen addition" OR "nitrogen amendment" OR "nitrogen enrichment" OR "nitrogen fertilization" OR "nitrogen deposition" OR "nitrogen load*"; (ii) "gene" AND "soil" AND "*PCR"; and (iii) "**amoA*" OR "*AOA*" OR "*AOB*" OR "*nirK*" OR "*nirS*".

Studies were selected if: (1) gene abundances in topsoil (0–20 cm) were quantified by quantitative real-time polymerase chain reaction (qPCR); (2) ambient and N loading treatments were conducted at the same experimental site under field conditions; (3) N loading duration lasted 1 year at minimum; (4) standard deviations and replicate numbers could be acquired. Ultimately, 101 eligible studies were included into our database (Zhang et al., 2021). For each study, we only included the observations comparing ambient and N loading treatments. When a study repeatedly measured gene abundances over time, we preferentially chose the measurements from the growing season or/and the last measurements (Chen et al., 2020). The flowchart of preferred reporting items for systematic reviews and meta-analyses (PRISMA) can be found in Supplementary Materials and Methods. The global distribution of N loading experiments is presented in Figure 1.

2.2 | Potential nitrification, potential denitrification, and soil N_2O emission

To investigate the potential linkages between soil N_2O emission and ammonia oxidizers and denitrifiers, we simultaneously tabulated potential nitrification, potential denitrification, and soil N_2O emission if available. Potential nitrification was estimated from the maximum rate of nitrate or nitrite production under optimal conditions (Hazard et al., 2021), while potential denitrification was calculated based on N_2O concentration in gas samples under anaerobic conditions and with addition of a readily available C source and nitrate (Philippot et al., 2007; Tang et al., 2019; Zhang et al., 2017). Soil N_2O emission was measured by static chambers followed by gas chromatography (Abalos et al., 2020; Hartmann et al., 2013). To reduce the bias of N_2O emission estimation, the studies that measured N_2O fluxes for less than 3 months were excluded (Li et al., 2020). We recorded the sampling time, frequency, and duration (seasonal or annual) of N_2O gas, and the average or cumulative estimation of N_2O emission. The calculation of relative treatment effect (i.e., response ratio) was independent of the unit of measurement (Deng et al., 2020; Hartmann et al., 2013). Furthermore, *ex situ* soil N_2O emission that measured by laboratory incubation was collected for exploratory analysis. Please see detailed information in Data S4.

2.3 | Environmental and experimental variables

To explore the key moderators of the effects of N loading on ammonia oxidizer abundance, denitrifier abundance, and soil N_2O emission, we recorded a broad range of environmental and experimental variables: latitude (27.72°S–64.02°N), longitude (126.80°W–153.02°E), elevation (1–3650 m), mean annual precipitation (MAP; 42–1899 mm), mean annual temperature (MAT; −0.5 to 28.0°C), soil clay content (1%–79%), soil pH (3.7–9.5), soil C:N ratio (4.89–23.41), N loading

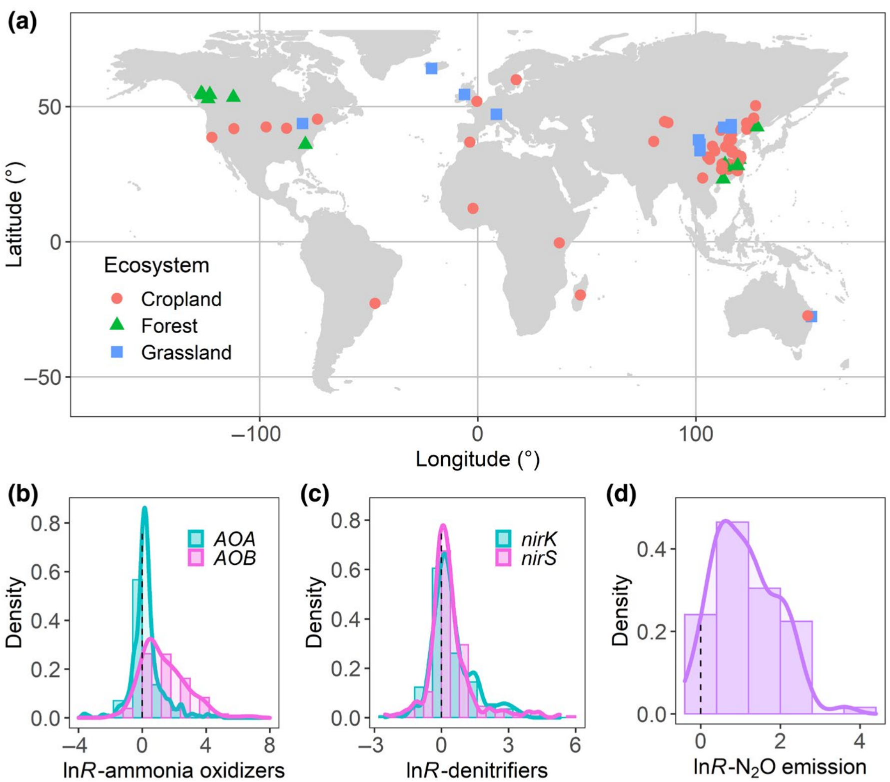

FIGURE 1 Overview of the data included in this meta-analysis. (a) Global distribution of N loading experiments in croplands, grasslands, and forests. Density distributions of the response ratios (ln*R*) of (b) ammonia oxidizer abundance, (c) denitrifier abundance, and (d) soil N_2O emission

duration (1–79 years), N loading rate (1.5–87.0 g N m^{-2} $year^{-1}$), ecosystem types (cropland, grassland, and forest), and N loading forms. Specifically, N loading forms were grouped into mineral N (e.g., urea, NH_4NO_3, and calcium nitrate), organic N (e.g., compost, manure, and biofertilizer), and mixed use of mineral and organic N. The data shown in figures were digitized using Grapher™ (https://www.goldensoftware.com/). When not reported, we extracted MAT and MAP from WorldClim 2.1 (https://www.worldclim.org/), and soil clay content, soil pH, and soil C:N from SoilGrid 2.0 (https://soilgrids.org/).

2.4 | Meta-analysis, model selection analysis, and regression analysis

The effects of N loading on ammonia oxidizer abundance, denitrifier abundance, potential nitrification, potential denitrification, and soil N_2O emission were assessed by calculating the natural logarithmic response ratio (ln*R*) and its variance for each observation (Hedges et al., 1999). Based on our preliminary statistical analysis, primer selections, and inhibition tests had no significant impacts on the ln*R* of gene abundances (Table S1). The overall effect size was estimated in a weighted mixed-effects model using "rma.mv" function from R package "*metafor*" (Viechtbauer, 2010). There were several studies that contributed more than one paired observation, because each of them designed multiple treatments, for example, different study sites, N loading rates, or/and forms. To ensure the independence of each observation, we thus considered "study" and "observation" as random factors in the mixed-effects models. For the sake of data interpretation, the overall effect size was converted into the percentage change, that is, $(e^{\ln R} - 1) \times 100\%$. The overall effect of N loading on each response variable was considered significant if $p < .05$.

We used model selection analysis in the R package "*glmulti*" to identify the important predictors of the ln*R* of ammonia oxidizer and denitrifier abundances (Calcagno & de Mazancourt, 2010). The main advantage of this model selection analysis is that various kinds of numeric and non-numeric variables can be simultaneously evaluated, which can help explore the essential predictors. This model selection analysis was based on maximum likelihood estimation, fitting of all possible models containing the potential predictors. The relative importance of each predictor was calculated by the sum-of-Akaike-weights for all potential models that included this predictor. This value indicated the overall support of each predictor across all possible models. A cutoff of 0.8 was chosen to differentiate between important and non-essential predictors (Chen et al., 2018; Terrer et al., 2016). All available predictors (i.e., latitude, longitude, elevation, MAP, MAT, soil clay content, soil pH, soil C:N, ecosystem type, and N loading form, rate, and duration) were incorporated into the model selection analysis.

Regarding soil N_2O emission, relatively small sample size ($n = 58$) limited its applicability in the model selection analysis. Therefore, regression analysis was used to explore the relationships between the ln*R* of soil N_2O emission and the ln*R* of ammonia oxidizer and denitrifier abundances. To further understand how abiotic predictors influence the ln*R* of soil N_2O emission, we first evaluated the impacts of discrete variables (i.e., ecosystem type and N loading form) by using the test of moderators in R package "*metafor*". In regard to continuous variables, regression analysis was performed to fit the relationships between the ln*R* of soil N_2O emission and these variables (i.e., latitude, longitude, elevation, MAP, MAT, soil clay content, soil pH, soil C:N, and N loading rate and duration). The optimal regression model was chosen by Akaike information criterion (linear and quadratic models were considered). The predictor was considered important if $p < .05$. On the basis of the identified important predictors, we used R package "*lm*" to structure a multiple regression model for soil N_2O emission.

3 | RESULTS

Overall, N loading increased ammonia oxidizer abundance by 107% and denitrifier abundance by 45% ($p < .001$; Figure 2c,d). Model selection analyses identified that N loading form was the only predictor that exceeded the 0.8 sum-of-Akaike-weights cutoff for both microbial guilds (Figure 2a,b). Organic N loading induced greater increases in ammonia oxidizer and denitrifier abundances than mineral N loading ($p < .001$; Figure 2c,d and Table S2). Specifically, mineral and organic N loadings increased ammonia oxidizer abundance by 85% and 123%, and increased denitrifier abundance by 21% and 91%, respectively. In addition, the ln*R* of ammonia oxidizer abundance increased with N loading rate, while the ln*R* of denitrifier abundance were positively related to soil C:N and N loading rate ($p < .001$; Figure S1).

Nitrogen loading stimulated potential nitrification by 79% ($p < .001$), potential denitrification by 46% ($p = .010$; Figure S2a) and soil N_2O emission by 261% ($p < .001$; Figure 4a). The ln*R* of potential nitrification increased with the ln*R* of ammonia oxidizer abundance, and the ln*R* of potential denitrification raised with the ln*R* of denitrifier abundance ($p < .001$; Figure S2b,c). However, the ln*R* of soil N_2O emission were independent of the ln*R* of ammonia oxidizer and denitrifier abundances, which were true even within each subgroup database ($p > .05$; Figure 3 and Figure S3). Meanwhile, there was no clear relationship between the ln*R* of soil N_2O emission and the ln*R* of potential nitrification and potential denitrification ($p > .05$; Figure S4).

The test of moderators and regression analysis confirmed that ecosystem type, mean annual precipitation, soil pH, and soil C:N were important predictors of the ln*R* of soil N_2O emission (Table S3). For ecosystem type, N loading increased soil N_2O emission by 185% in croplands, 347% in grasslands, and 591% in forests ($p < .001$; Figure 4a). The ln*R* of soil N_2O emission showed a quadratic relationship with mean annual precipitation ($p = .002$; Figure 4b). In addition, the ln*R* of soil N_2O emission decreased with soil pH ($p = .007$; Figure 4c) and increased with soil C:N ($p = .019$; Figure 4d). Based on these four identified important predictors, a multiple regression model for soil N_2O emission was structured: $\ln R\text{-}N_2O \sim \text{Ecosystem} + MAP^2 + pH + C{:}N$ ($n = 58$, $p = .007$, $R^2 = .285$).

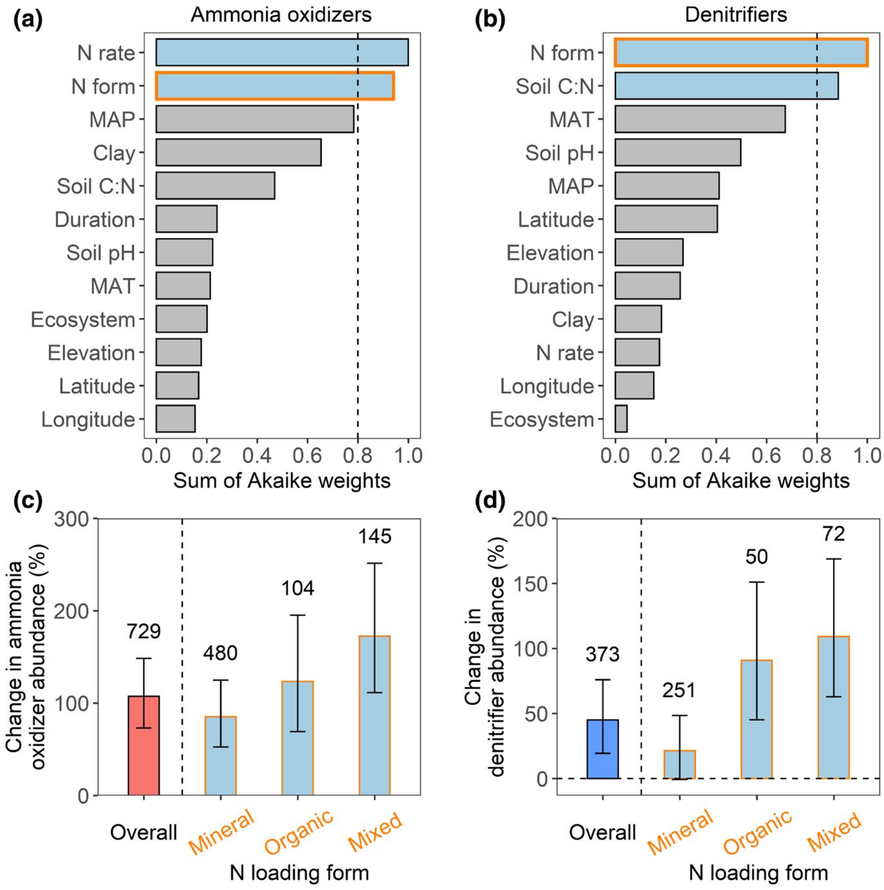

FIGURE 2 The effects of N loading on ammonia oxidizer and denitrifier abundances. (a) and (b) Model selection analyses identified that N loading form was the only predictor that exceeded the 0.8 sum-of-Akaike-weights cutoff for both microbial guilds. The dashed line shows a cutoff of 0.8 to distinguish important predictors. The effects of N loading on (c) ammonia oxidizer and (d) denitrifier abundances grouped by various N loading forms. Error bars show 95% confidence intervals, and the numbers above the error bars indicate sample sizes. MAP, mean annual precipitation; MAT, mean annual temperature

4 | DISCUSSION

Nitrogen loading substantially increased soil ammonia oxidizer and denitrifier abundances (Figure 2). External N inputs alleviate soil N limitation, supporting the growth and activity of ammonia oxidizers and denitrifiers (Levy-Booth et al., 2014). Both microbial guilds increased more at higher rates of N loading (Figure S1a,c), as substrate availability is a crucial factor for microbial growth (Stein, 2020). Organic N loading had larger effects on ammonia oxidizer and denitrifier abundances than mineral N loading. On the one hand, organic N loading (e.g., manure and compost) develops more favorable growth conditions for ammonia oxidizers and denitrifiers (Luo et al., 2018; Ollivier et al., 2011). For example, the accompanied C inputs with organic N loading provide C as an energy sources to support heterotrophic denitrifiers (Tatti et al., 2013). In another example, some ammonia oxidizers produce ammonia (as their substrate) by degrading organic N compounds via enzymes, for example, urease and cyanase (Kuypers et al., 2018). On the other hand, organic N loading modifies soil pH by increasing base cation inputs, whereas mineral N loading significantly decreases soil pH (Raza et al., 2020; Zeng et al., 2017). Soil acidification and the potential toxic effects that caused by mineral N loading would weaken the positive responses of ammonia oxidizer and denitrifier abundances to N loading (Song et al., 2020; Zhao et al., 2020). In addition to N loading rate and form, the responses of denitrifier abundance were positively related to soil C:N (Figure S1b), which might be associated with the heterotrophic strategy of denitrifiers (Philippot et al., 2007).

There was no clear relationship between the responses of ammonia oxidizer and denitrifier abundances and the responses of soil N_2O emission, despite their abundances being positively correlated with potential nitrification and potential denitrification (Figure 3, Figures S2 and S3). This suggests that the linkages between microbial guild abundances and potential nitrification and potential denitrification do not necessarily translate into effective prediction capacity for soil N_2O emission under N loading. Three reasons may account for the poor predictability of shifts in ammonia oxidizer and denitrifier abundances to changes in soil N_2O emission (Figure 5). First, a portion of N_2O produced by ammonia oxidizers and denitrifiers is converted into N_2 via N_2O-reducers (Kuypers et al., 2018). This explanation is in line with the positive relationship between soil N_2 emission and *nosZ*-I abundance (Figure S5b). In addition, *nosZ*-II also plays important role in N_2O reduction, whereas it was not considered in this study due to the paucity of data. Further data availability (e.g., *nosZ*-II gene) and refinements in the categorization of microbial guilds (e.g., functional gene ratios, diversity metrics) are needed before we can inform soil biogeochemical models with N-cycling functional gene data (Levy-Booth et al., 2014; Shan et al., 2021).

Second, the confounding impacts of abiotic factors potentially hinder the applicability of ammonia oxidizer and denitrifier abundances as effective predictors of soil N_2O emission (Graham et al., 2014; Levy-Booth et al., 2014; Pärn et al., 2018). Our results showed

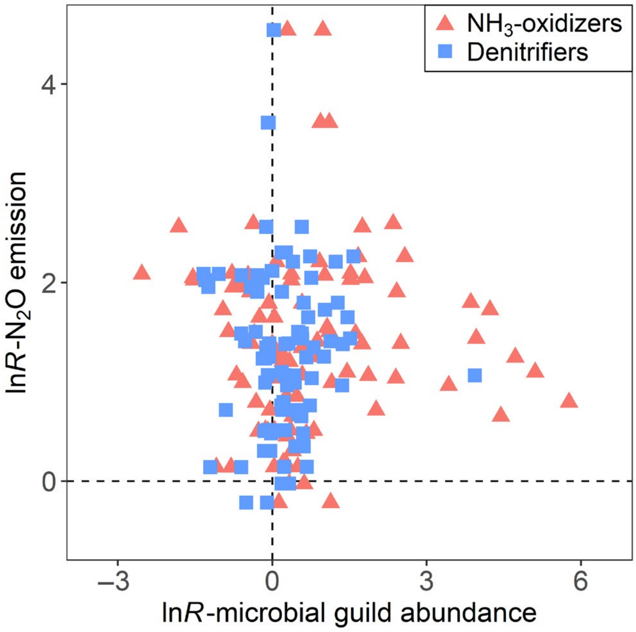

FIGURE 3 The relationships between the response ratios (ln$R$) of soil N_2O emission and the ln$R$ of microbial guild abundances. ln$R$-N_2O emission versus ln$R$-ammonia oxidizer abundance ($n = 98$, $p = .950$, $R^2 < .001$). ln$R$-N_2O emission versus ln$R$-denitrifier abundance ($n = 91$, $p = .702$, $R^2 = .002$). The n, p, and R^2 are statistic values of the optimal regression model chosen by Akaike information criterion

that mean annual precipitation, soil pH, and soil C:N were among the major abiotic factors affecting the responses of soil N_2O emission to N loading (Figure 4). Precipitation affects soil moisture and oxygen availability, and both are closely related to soil N_2O emission (Saggar et al., 2013; Song et al., 2019). The quadratic relationships between soil N_2O emission and water-filled pore space were observed in other studies (Bouwman, 1998; Ciarlo et al., 2007; Dalal et al., 2003), reflecting intermediate soil moisture at which soil N_2O emission from both nitrification and denitrification was favored. Soil pH regulates microbial structure and functions as well as substrate speciation and chemical reactions (Abalos et al., 2020; Su et al., 2019), leading to the modification of N_2O:N_2 emission ratio (Bakken et al., 2012; Čuhel et al., 2010). For example, soil acidification decreases N_2O-reductase activity and electron-transfer efficiency (Su et al., 2021), which suppresses N_2 production thereby enhancing the fraction of N_2O emission (Čuhel et al., 2010; Wang et al., 2018). The C:N is the indicator of soil quality, and a high C:N often indicates N limitation (Terrer et al., 2016). It was reported that N_2O emission in N-limited soils had stronger responses to N loading than in C-limited soils (Deng et al., 2020). As a result, the sensitivity of soil N_2O emission to N loading across different ecosystems would likely vary with local precipitation, soil pH, and soil C:N.

Lastly, several understudied mechanisms would also contribute to soil N_2O emission, for example, fungal denitrification (Aldossari & Ishii, 2021) and chemical processes (Chalk & Smith, 2020; Zhu-Barker et al., 2015). For example, recent studies have identified that

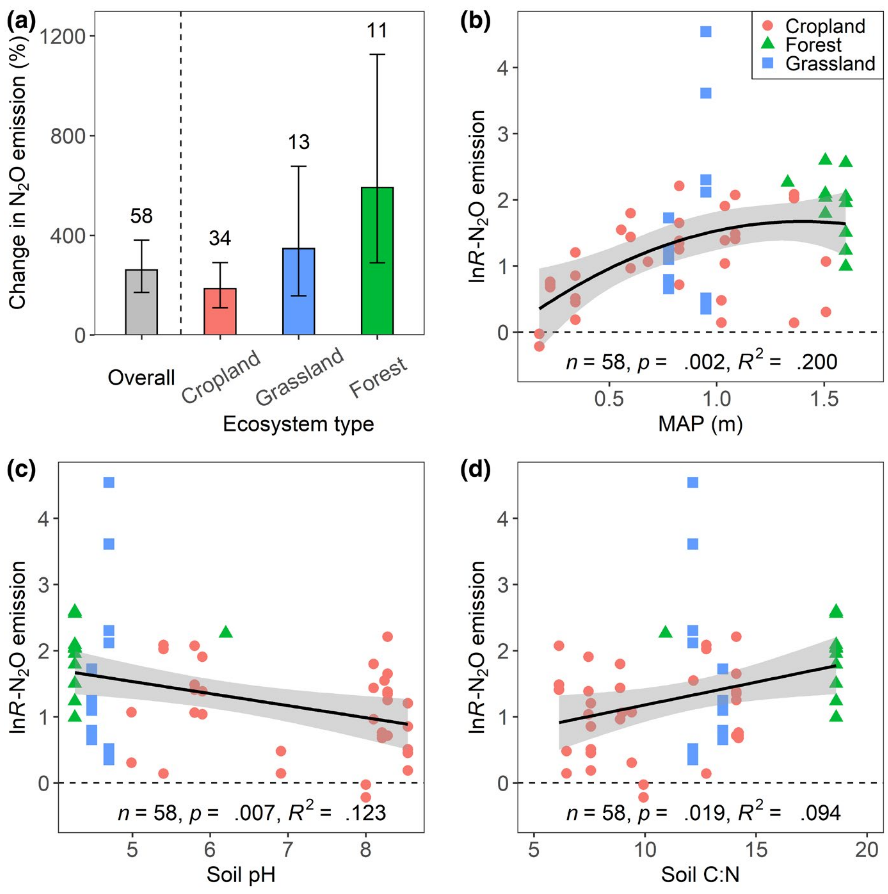

FIGURE 4 Key abiotic predictors for the response ratios (ln$R$) of soil N_2O emission. (a) The effects of N loading on soil N_2O emission grouped by different ecosystem types (the test of moderators: $n = 58$, $p = .024$, $F = 3.991$). Error bars show 95% confidence intervals, and the numbers above the error bars indicate sample sizes. The relationships between the ln$R$ of soil N_2O emission and (b) MAP, (c) soil pH, and (d) soil C:N. The n, p, and R^2 are statistic values of the optimal regression model chosen by Akaike information criterion. MAP, mean annual precipitation

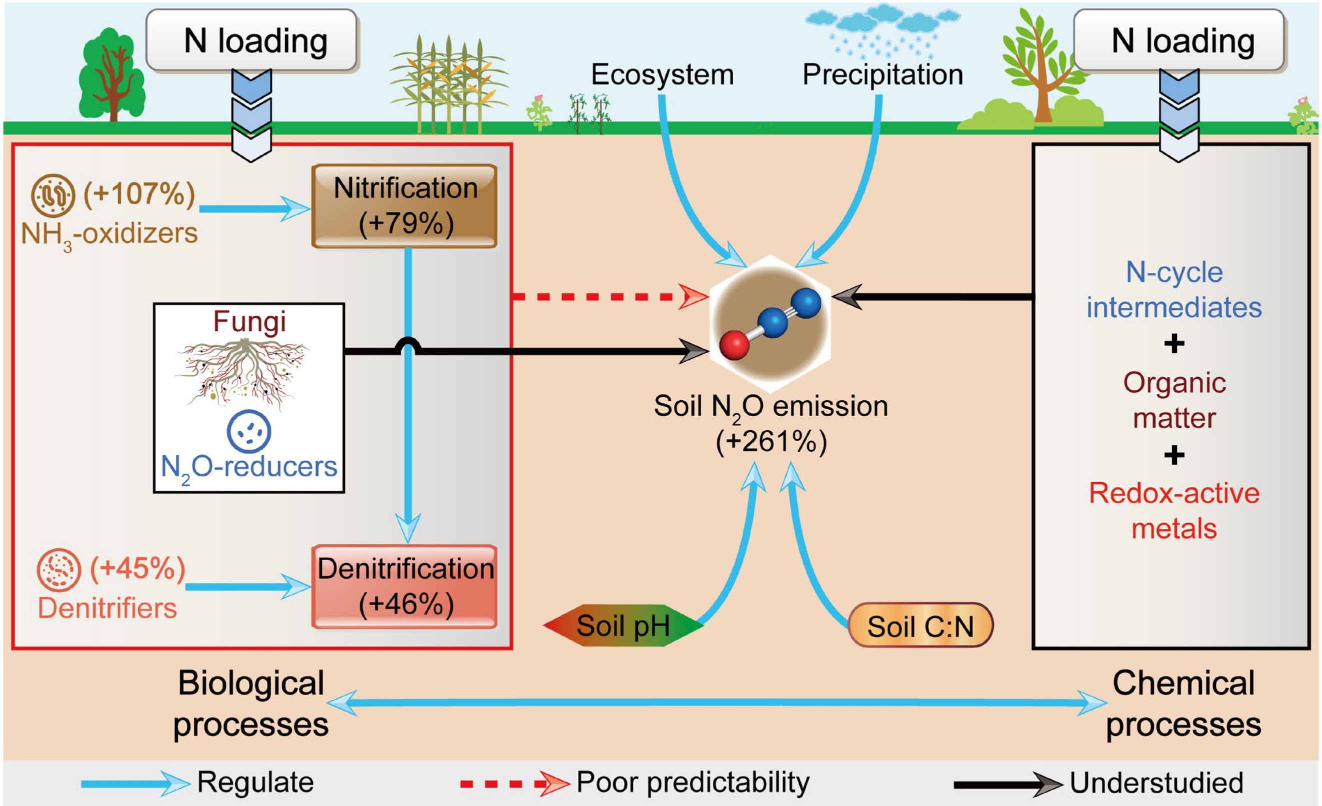

FIGURE 5 Schematic diagram of potential mechanisms underlying the effects of N loading on soil N_2O emission. Possible reasons for poor predictability of changes in soil N_2O emission from shifts in ammonia oxidizer and denitrifier abundances include: (1) N_2O consumption by N_2O-reducers community; (2) the confounding impacts of abiotic factors; and (3) the contribution of fungal denitrification and chemical processes to soil N_2O emission

certain fungi (e.g., *Fusarium*) can directly produce N_2O (Aldossari & Ishii, 2021), and plant mycorrhizal associations can indirectly influence soil N_2O emission by structuring N-cycling microbiomes (Mushinski et al., 2021). Moreover, the lack of relationship between the responses of soil N_2O emission and the responses of potential nitrification and potential denitrification (Figure S4) partially supports the hypothesis that biological processes are not the sole contributors to soil N_2O emission (Zhu-Barker et al., 2015). Indeed, emerging studies have confirmed that N_2O gas can also be emitted through a range of chemical processes, for example, the non-enzymatic reactions between N cycle intermediates (hydroxylamine, nitrous acid, nitric oxide, and nitrite), redox-active metals (iron and manganese), and soil organic matter (Chalk & Smith, 2020; Zhu-Barker et al., 2015). Although biological processes might be the main sources of soil N_2O emission (Prosser et al., 2020; Stein, 2020), the contribution of chemical processes warrants further investigation.

Our results showed that ammonia oxidizer and denitrifier abundances were poor predictors of soil N_2O emission under N loading at the global scale. This finding challenges the earlier hypothesis that N stimulation of ammonia oxidizer and denitrifier abundances would directly cause higher soil N_2O emission (Hu et al., 2015; Linton et al., 2020; Morales et al., 2010; Ouyang et al., 2018). In fact, empirical support for the direct linkages between soil N_2O emission and ammonia oxidizer and denitrifier abundances under N loading was mostly observed in laboratory experiments under well-controlled conditions, for example, specific model microorganism, pH, temperature, moisture, and substrate availability (Hink et al., 2018; Jones et al., 2014; Qiu et al., 2019). This explanation was strengthened by the significant correlation between *ex situ* soil N_2O emission and denitrifier abundance (Figure S6), corroborating the potential gaps between *in situ* and *ex situ* measurements of soil N_2O emission. Furthermore, we found that key abiotic factors (e.g., precipitation and soil pH) explained N-induced changes in soil N_2O emission at the global scale. Our findings indicate that relatively coarse-scale and easy to obtain measures of abiotic factors can be used to understand global responses of soil N_2O emission to N loading. This contention supports the current use of abiotic factors rather than microbial abundance for model simulations and potential identification of regional hotspots of N-induced soil N_2O emission across the world (Tesfaye et al., 2021; Yang et al., 2021).

The methodological caveats of the base studies synthesized in this meta-analysis do not compromise our analyses of the key drivers of N-induced soil N_2O emission, but highlight the key guidelines for future research. First, there may be some unaccounted biases, for example, unbalanced samples across ecosystem types, a bias toward the temperate biome, and a relatively small sample size of soil N_2O emission. It will advance the field if more similar studies can be conducted in underrepresented areas, for example, tropical biome, grassland, and forest ecosystems. Second, DNA-based qPCR for four specific marker genes cannot fully capture the complete view of microbial N-cycling community, since several other biological pathways (e.g., fungi and *nosZ*-II) are known to be important but not captured. The state-of-the-art metagenomic technologies may capture a greater gene diversity (Chen & Sinsabaugh, 2020), reflecting more accurately changes in microbial N-cycling community. Lastly, manual measurements by static chambers may miss some important N_2O emission pulses. Advanced automatic chambers will improve the analysis of N_2O emission pulses, as it permits N_2O measurements with a high temporal resolution. Despite these potential limitations, by using available databases and methods, we demonstrate the greater importance of key abiotic factors in driving N-induced changes in soil N_2O emission than ammonia oxidizer and denitrifier abundances. A thorough understanding of the influences of abiotic factors on soil N transformations can be a research priority for optimizing fertilization regimes to mitigate N-induced soil N_2O emission.

5 | CONCLUSIONS

This work points that although ammonia oxidizer and denitrifier abundances being positively related to potential nitrification and potential denitrification, how N loading affects their abundances is a poor predictor of changes in soil N_2O emission at the global scale. Therefore, the studies may overestimate the predictability of ammonia oxidizer and denitrifier abundances to soil N_2O emission under field conditions if merely based on the laboratory-based direct linkages between soil N_2O emission and microbial guild abundances. Indeed, this study identifies the greater power of key abiotic factors (e.g., precipitation and soil pH) in explaining N-induced changes in soil N_2O emission, at least compared with the shifts in ammonia oxidizer and denitrifier abundances by a specific molecular approach that captures an important but incomplete view of the microbial N-cycling community. Our synthesis underlines the poor ability of ammonia oxidizer and denitrifier abundances to predict N-induced soil N_2O emission under a broad range of pedoclimatic conditions. As such, caution is required when extrapolating the laboratory-based direct linkages between soil N_2O emission and microbial guild abundances into Earth system models.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

YZ, FZ, and JC designed the study and conducted the data analysis. YZ collected the data and wrote the initial manuscript. YZ, FZ, DA, YL, DH, BAH, PGP, YK, JEO, UJ, and JC collaborated on data interpretation. All authors substantially contributed to revisions.

DATA AVAILABILITY STATEMENT

The data are available from Figshare (https://doi.org/10.6084/m9.figshare.14370896).

ORCID

Yong Zhang https://orcid.org/0000-0002-1181-032X
Feng Zhang https://orcid.org/0000-0002-6866-1468
Diego Abalos https://orcid.org/0000-0002-4189-5563
Yiqi Luo https://orcid.org/0000-0002-4556-0218
Dafeng Hui https://orcid.org/0000-0002-5284-2897
Bruce A. Hungate https://orcid.org/0000-0002-7337-1887
Pablo García-Palacios https://orcid.org/0000-0002-6367-4761
Yakov Kuzyakov https://orcid.org/0000-0002-9863-8461
Jørgen Eivind Olesen https://orcid.org/0000-0002-6639-1273
Uffe Jørgensen https://orcid.org/0000-0002-5930-3124
Ji Chen https://orcid.org/0000-0001-7026-6312

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