



How climate and soil properties affect the abundances of nitrogen-cycling genes in nitrogen-treated ecosystems: a meta-analysis

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Received: 28 September 2021 / Accepted: 31 March 2022 / Published online: 14 April 2022
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Abstract

Purpose The abundance of nitrogen (N)-cycling genes is frequently used to indicate N cycling and predict N₂O emissions. However, it remains difficult to clearly define how soil N-cycling genes in different ecosystems respond to anthropogenic N additions.

Methods We applied a meta-analysis approach to examine data about N-cycling genes (*nifH*, ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB), *nirK*, *nirS*, and *nosZ*) in different ecosystems from 119 peer-reviewed articles.

Results In the ecosystems examined, the patterns of change in the abundances of the target genes, apart from AOA, varied considerably. This variation reflects the distinctive soil characteristics of ecosystems that develop when different forms of N are applied at different rates and over different durations. The *nifH* abundance decreased significantly, by 32.79%, in forests but did not change in grasslands and croplands. The AOB abundance increased

in all three ecosystems, by 193.06% in grasslands, 73.26% in forests, and 151.86% in croplands, respectively. The denitrification gene abundances, namely the *nirK*, *nirS*, and *nosZ*, in croplands also increased significantly, by 60.74%, 47.42%, and 69.54%, respectively.

Conclusion In general, climate factors and long-term applications of organic N at high rates had significant effects on the gene abundances in different ecosystems, through their influence on soil properties. An enhanced understanding of how N additions influence the abundance of other N-cycling functional genes can help us improve our ability to model the populations and activities of microbial functional communities and predict N fluxes.

Keywords Cropland · Forest · Grassland · Meta-analysis · Nitrogen addition · Nitrogen-cycling functional gene

Responsible Editor: Luca Bragazza.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11104-022-05420-6>.

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Introduction

Nitrous oxide (N₂O) is a by-product of the complex microbial nitrogen (N) cycle in the environment. The element N undergoes the processes of N-fixation, nitrification, and denitrification, thus producing N₂O (Canfield et al. 2010; Kuypers et al. 2018). These reactions are mainly driven by the physiological metabolic activities of microorganisms in the soil. Soil microorganisms are susceptible to external

interference, especially applications of chemical and organic N fertilizers, which change the N availability in soil and substantially influence the biochemical processes of the N cycle (Makowski 2019). Therefore, the responses of soil microorganisms to external N additions can strongly influence N-cycling processes (Ouyang et al. 2017).

During N-cycling processes, microorganisms synthesize corresponding enzymes through the expression of specific genes. The nitrogen fixation gene (*nifH*) encodes nitrogenase reductase and reduces nitrogen gas (N_2) to ammonium (NH_4^+). The nitrification genes (AOA and AOB) synthesize ammonium monooxygenase and promote the transformation of ammonia (NH_3) or NH_4^+ to hydroxylamine, which then undergoes further cycling. Denitrification involves the reduction of nitrate (NO_3^-) to nitrite (NO_2^-). After NO_3^- is reduced to NO_2^- , it may be partitioned along two pathways, either to generate NH_4^+ by dissimilatory reduction, or to generate N_2O (Canfield et al. 2010). Thus, various denitrification genes have important roles in regulating N_2O emissions and, depending on the N management approach, the resulting N_2O emissions will vary. *NirS* and *nirK* indicate denitrifiers that convert NO_2^- to nitric oxide (NO), while *nosZ* encodes nitrous oxide reductase and then the enzyme catalyzes the transformation of N_2O to N_2 , the final step of denitrification (Canfield et al. 2010). These functional genes are frequently used to describe the richness and diversity of microbial communities in specific N transformation processes (Gaby and Buckley 2012; Henry et al. 2006; Morales et al. 2010; Ouyang et al. 2018).

Many scholars have studied how and why the abundances of N-cycling genes change under N additions. Different ecosystems have unique soil characteristics, which impact the gene abundance. Carey et al. (2016) determined that, in different ecosystems (croplands, pastures, and unmanaged wildlands), nitrification genes, namely ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), all responded differently to N fertilizer, and that N applications had most effect on AOA in croplands and on AOB in wildlands. Also, different N forms have different effects. When organic N is applied, carbon (C) inputs are higher than under inorganic N applications, which then increases the metabolic activity of microorganisms and the gene abundance (Schmidt et al. 2011; Sulman et al. 2014). Sulman et al. (2014) found

that microorganisms had differing abilities to obtain C and that the gene abundances differed among ecosystems. Carey et al. (2016) reported that the N addition rates and the duration of the N additions (the period from the start of the N application to the end) could affect ammonia oxidizers in different ecosystems. Furthermore, environmental factors may have a strong influence on N additions. For example, under high rates of atmospheric N deposition, the available N in soil may increase. Under the increased levels of available N, the soil microbial biomass and respiration may be suppressed (Phillips and Fahey 2007; Yang et al. 2021), which will in turn affect the microbial composition and the metabolic potential (Hallin et al. 2009; Yang et al. 2021; Zhao et al. 2020). High N inputs can cause increases in the soil exchangeable aluminium (Al^{3+}) content and decreases in the soil pH (Bowman et al. 2008; Van Breemen et al. 1983). The aluminium will have toxic effects on microorganisms (Kuperman and Edwards 1997) and may also affect the expression of functional genes (Sun et al. 2021). Zhang et al. (2021) also reported that the abiotic factors played key roles in regulating the ammonia oxidizer and denitrifier abundances under N loading.

The findings discussed so far are from field experiments or indoor cultivation experiments that were performed in small areas for specific research purposes. The results of these studies are inconsistent, so researchers have carried out meta-analyses of the data from the individual studies to identify overall patterns from the different studies. In their meta-analysis, Carey et al. (2016) examined 98 sets of measurement data from 33 articles to gain insights into how N additions affected the abundances of AOA and AOB during nitrification. While they found that AOB responded more positively than AOA, they did not explore other functional genes or environmental factors that could have affected the gene abundances in their analysis. Ouyang et al. (2018) explored how the abundances of N cycling genes changed in agricultural ecosystems treated with N fertilizers. They found that, apart from *nifH*, the functional gene abundances increased during nitrification (*amoA*) and denitrification (*nirK*, *nirS*, and *nosZ*) when fertilized with N. Ouyang et al. (2018) concluded that the N form and the soil pH were the main drivers behind the responses of functional genes to N additions. Data from numerous studies and the World Bank, (<https://data.worldbank.org.cn/indicator>), show that

grassland, forest, and cropland, which account for a large proportion of terrestrial ecosystems worldwide, suffer from over-applications of N (Bonan 2008; Hovenden et al. 2019; IPCC, 2013). An analysis that compares N-cycling genes among different N-treated ecosystems, and highlights how they are related to soil properties, will provide support for evaluations of gene-based models of N cycling in soil.

In this study, we carried out a meta-analysis based on 397 field observations from 119 articles related to N-cycling genes in grassland, forest, and cropland ecosystems. The aim of the analysis was to (1) compare the responses of functional genes in different ecosystems to N additions, and (2) identify the key drivers of change in the gene abundances and how they affected the responses of N-cycling functional genes to N additions in different ecosystems.

Materials and methods

Data extraction and collection

To obtain the data for this analysis, we searched for studies about N-cycling functional genes published before February 2022 under different keyword combinations (nitrogen fixation, ammonia oxidizing, nitrous oxide, functional gene, grassland, forest, wetland, and agriculture) in Google Scholar, the Web of Science, and the China Knowledge Resource Integrated Database (CNKI). We carried out a detailed examination of studies that reported the effects of N additions on N-cycling functional genes in soil and included those that met the following criteria: (1) the authors used real-time quantitative PCR (qPCR) to measure the abundance of functional genes; (2) the experiment had an established control group without N applications; (3) they were field studies; (4) at least one functional gene was determined; (5) the sample size, mean values, and standard errors or standard deviations of the experimental and control groups were provided in the article or could be calculated; (6) data with less than three replicates were excluded, and (7) the data could be obtained either directly from the text or through GET DATA GRAPH DIGITIZER software (version 2.26, <http://www.getdatagraph-digitizer.com/download.php>).

We conducted a meta-analysis of the following functional genes where there were sufficient

observations: *nifH*, *amoA* (AOA and AOB), *nirK*, *nirS*, and *nosZ*. For studies without standard deviations or standard errors, we referred to other data sets to establish *log* equations for estimating the standard deviation and the mean of the experimental results (Marinho et al. 2003). For a meta-analysis, data must be independent of each other; we therefore selected only the data measured during the latest year from multi-year experiments, and for the top layer of the soil where an experiment involved adding the same treatment to different soil layers. To increase the research data, we also included multi-factor research results. For example, two treatments of phosphorus-potassium (PK) additions and nitrogen-phosphorus-potassium (NPK) additions were set up in an individual study. We used the PK treatment as the control group, and the NPK treatment as the experimental group.

As well as collating data for the gene abundance, we also recorded data for the mean annual temperature (MAT), the mean annual precipitation (MAP), the soil pH, the soil water content (WC), soil available phosphorus (AP), NH_4^+ , NO_3^- , soil organic carbon (SOC), total nitrogen (TN), soil microbial carbon (MBC), soil microbial nitrogen (MBN), and the ratio of soil organic carbon to soil total nitrogen (C/N). We also collected 73 observations of nitrification potential (NP) and 41 observations of N_2O emissions, but there were insufficient data to permit robust analysis of each ecosystem. Overall, we collected 397 sets of observation data from 119 articles about N-cycling genes. Of these, 86 observations were from grassland, 57 were from forest, and 253 were from cropland (Fig. S1). To study the influence of other experimental or environmental factors on the functional gene abundance, we grouped the data into (1) ecosystem type (grassland ecosystems, forest ecosystems, and cropland ecosystems); (2) N form (inorganic, organic, and inorganic–organic mixed N; inorganic N forms included NH_4^+ , NO_3^- , NO_2^- , and urea, and organic N forms, including compost, crop residues, and animal manure); (3) N addition rate (with 100 and 200 kg N ha⁻¹ as the thresholds, giving different gradient divisions according to the amount of data and response ratios of each gene); and (4) N fertilization duration (with 5 and 10 years as the thresholds, resulting in different gradient divisions according to the amount of data and response ratios of each gene). For the two continuous variables, the N

rate, duration, and gradient were partitioned based on the observations at each level and the gene abundance responses to each gradient in the preliminary analysis, in line with previous studies (Carey et al. 2016; Liu and Greaver 2010; Ouyang et al. 2018).

Data analysis

We used the natural logarithm of the response ratio (RR) as the effect size of the amount of N added on the abundances of the different N cycling genes (Hedges et al. 1999):

$$RR = \ln\left(\frac{\overline{X_E}}{\overline{X_C}}\right) = \ln(\overline{X_E}) - \ln(\overline{X_C}), \quad (1)$$

where $\overline{X_E}$ and $\overline{X_C}$ represent the mean results of the experimental and control groups, respectively. The following formula was used to calculate the corresponding variance (v):

$$v = \frac{S_E^2}{N_E \overline{X_E}^2} + \frac{S_C^2}{N_C \overline{X_C}^2}, \quad (2)$$

where S_E and S_C represent the standard deviation of the experimental and control groups, and N_E and N_C represent the number of samples in the experimental and control groups, respectively.

We used the *rma* function in the *metafor* package (Viechtbauer 2010) to calculate the weighted effect size (RR_{++}) and the 95% confidence interval (CI) with the mixed-effects model. When the 95% CI overlapped with 0, the difference between the results of the experimental and control groups was not significant; when the 95% CI did not overlap with the invalid line, the average effect sizes were considered significant. To show the impact more clearly, we converted the effect size (RR_{++}) to a percentage expression:

$$\text{Equation} = (e^{RR_{++}} - 1) \times 100\% \quad (3)$$

The heterogeneity was calculated for each categorical group. We partitioned the total heterogeneity (Q_T) into the between-group heterogeneity (Q_M) and the residual error (Q_E). We used the Q_M to determine whether the values of the effects of the different groups differed noticeably. When $P < 0.05$, there was a significant difference in the response of

the functional genes to N additions among gradients or categories (Rosenberg et al. 2000).

The relative effects of the climate and soil factors on the responses of the functional gene abundances to N additions were examined by calculating a relative importance value for each variable. The relative importance value was calculated as the sum of the Akaike weights for all the models that included this factor using the corrected Akaike's Information Criteria with the *glmulti* package (Calcagno and de Mazancourt 2010). A cut-off of 0.8 was set for the most important predictors (sum of Akaike weights > 0.8) (Chen et al. 2020; Feng and Zhu 2019; Terrer et al. 2016). We used linear regression analysis to fit the relationships between the variables and calculated the corresponding R^2 and P values. We used the R platform [version 4.0.3] (<https://www.R-project.org>) to perform all the statistical analysis and GraphPad Prism 8 (<https://www.graphpad.com/>) to draw the plots.

Results

Effects of N additions on physical and chemical soil factors

In the different ecosystems, the responses of the physical and chemical soil properties to N additions differed significantly (Fig. 1). The N additions had significant effects on the soil pH in forests, croplands, and grasslands ($P < 0.05$), and the effect size was greatest for grasslands. The NH_4^+ and NO_3^- increased significantly across all the ecosystems; the effect size of NH_4^+ was greatest for forests and least for croplands, while the effect size of NO_3^- was greatest for grasslands and least for forests (Fig. 1). The N additions had significant effects on SOC and TN in croplands ($P < 0.05$), TN in forests ($P < 0.05$), but had no significant effects on TN or SOC in the grasslands ($P > 0.05$). The MBC and MBN had contrasting responses to the N additions across the ecosystems. The responses of MBC and MBN in croplands were positive and significant (MBC = 40%, MBN = 45%, respectively, ($P < 0.05$)) but were insignificant in forests ($P > 0.05$). MBN had a positive response, while MBC had insignificant response, in grasslands.

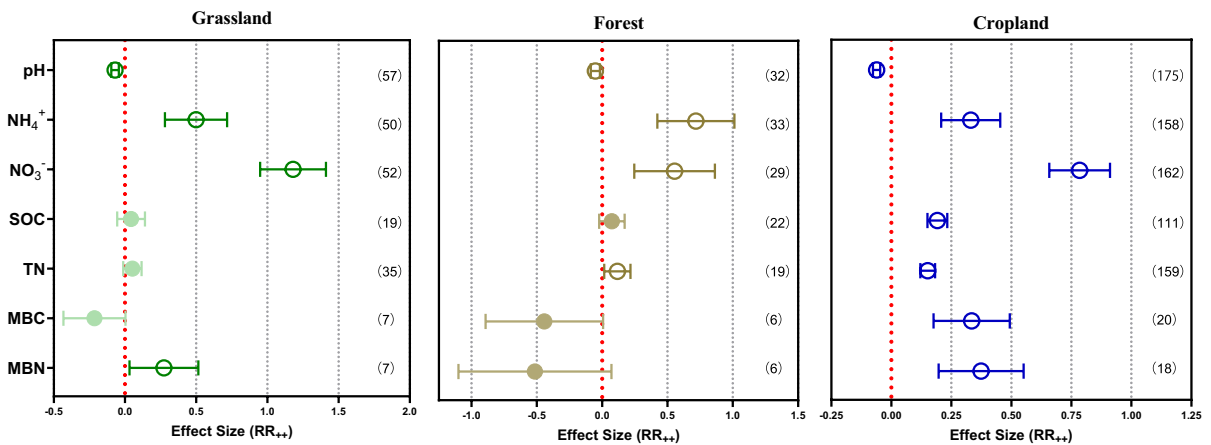


Fig. 1 Effect size of N additions on soil physical and chemical variables. Error bars represent the 95% confidence intervals. The results with significant responses are indicated by hollow dots. The numbers in parentheses represent the number of

studies included for each variable. SOC, soil organic carbon; TN, soil total nitrogen; MBC, soil microbial carbon; MBN, soil microbial nitrogen

Effects of N additions on N-cycling functional genes

The N application rate had a significant effect on the response of the *nifH* abundance in grasslands, but the N application duration and N form were

the main influences of change in forests and croplands, respectively ($P < 0.05$) (Fig. 2, Table 1). In grasslands, the *nifH* abundance increased significantly (by 58%) when the N application rate was low (<100 kg N ha⁻¹) but decreased by 45% when

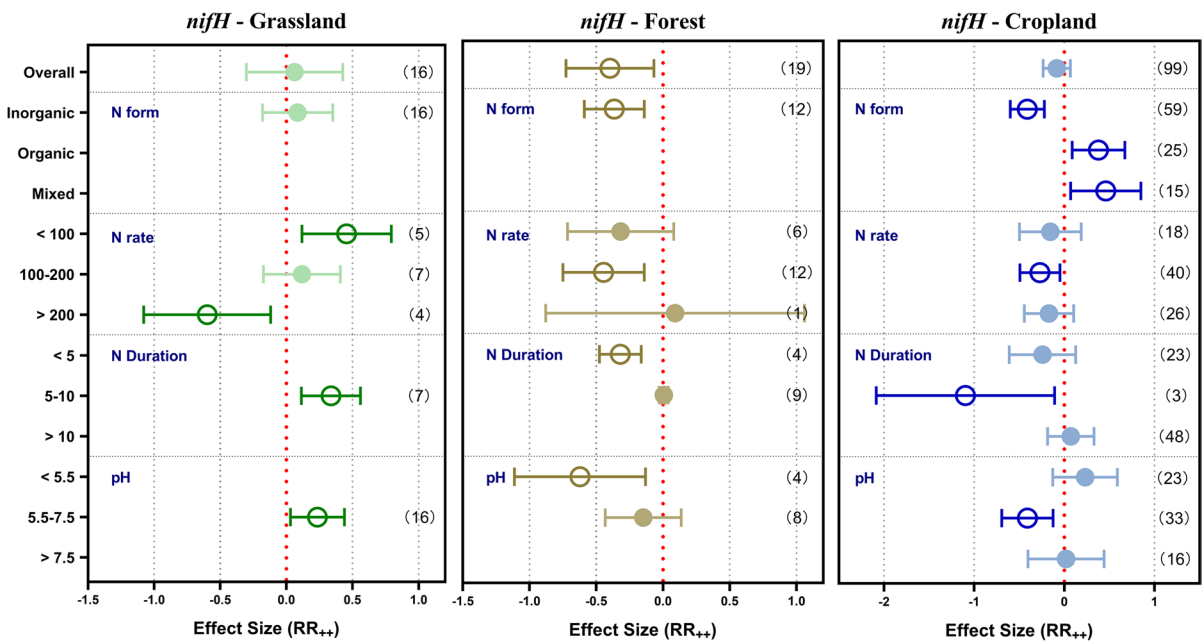


Fig. 2 The effect size of the N fixation gene (*nifH*) to N additions in different ecosystems. Error bars indicate the 95% confidence intervals. The results with significant responses are

indicated by hollow dots. The numbers in brackets indicate the number of studies included for each variable. ‘Overall’ indicates the abundance of each functional gene

the N application rate was high ($>200 \text{ kg N ha}^{-1}$) ($P < 0.05$). The *nifH* abundance in forests decreased significantly when the N application duration was less than 5 years (-27%). In croplands, the *nifH* abundance decreased under inorganic N applications but increased under organic N and mixed N applications ($P < 0.05$).

The response of AOA to N additions was mainly controlled by the N application duration in grasslands, and by the form of N and the pH in croplands ($P < 0.01$) (Fig. 3, Table 1). The AOA abundance in grasslands did not change significantly when the N application duration was less than 10 years ($P > 0.05$) but increased significantly when the N application duration exceeded 10 years ($P < 0.05$). In croplands, the AOA abundance decreased by 23% under inorganic N applications but increased by 99% under organic N applications ($P < 0.05$). When the pH was between 5.5 and 7.5, the AOA abundance

decreased by 27% ($P < 0.05$). The AOB abundance was not significantly affected by the different variables in forests and grasslands, but was significantly affected by the N form ($P < 0.01$) and the N application duration ($P = 0.013$) in croplands (Fig. 3, Table 1). The AOB abundance showed the greatest increase in croplands for mixed N applications and when the N application duration was greater than 10 years ($P < 0.05$).

The patterns of change in the denitrification genes varied significantly among the ecosystem types (Fig. 4, Table 1). *NirK* was mainly controlled by the N form in forests but was mainly controlled by the N form and N application duration in croplands. The *nirK* abundance increased significantly under inorganic N applications but decreased significantly under organic N applications in forests ($P < 0.05$). The *nirS* abundance in croplands was significantly affected by the N form

Table 1 Heterogeneity (Q_M) of the response of N-cycling functional genes to N additions for different variables in the different ecosystems

Variable	N form			N rate			N duration			pH		
	Q_M	df	P	Q_M	df	P	Q_M	df	P	Q_M	df	P
<i>Grassland</i>												
<i>nifH</i>	NA	NA	NA	12.397	2	< 0.01**	NA	NA	NA	NA	NA	NA
AOA	4.467	2	0.107	2.547	2	0.280	73.394	2	< 0.01**	1.005	2	0.605
AOB	5.369	2	0.068	3.765	2	0.152	2.123	2	0.346	1.163	2	0.559
<i>nirK</i>	0.053	2	0.974	5.466	2	0.065	4.019	2	0.134	0.071	1	0.790
<i>nirS</i>	0.702	2	0.704	1.487	2	0.476	0.923	1	0.337	0.133	1	0.715
<i>nosZ</i>	0.147	1	0.702	56.601	2	< 0.01**	2.195	2	0.334	NA	NA	NA
<i>Forest</i>												
<i>nifH</i>	NA	NA	NA	1.171	1	0.557	15.777	1	< 0.01**	2.672	1	0.102
AOA	3.389	1	0.066	0.619	2	0.734	0.329	2	0.849	0.051	1	0.821
AOB	0.069	1	0.792	3.048	2	0.218	0.239	2	0.888	0.537	1	0.464
<i>nirK</i>	6.484	1	0.011*	0.865	2	0.649	3.687	2	0.158	0.435	1	0.510
<i>nirS</i>	2.380	1	0.168	3.214	2	0.201	0.036	1	0.849	0.563	1	0.453
<i>nosZ</i>	4.817	1	0.028*	1.398	2	0.497	0.019	2	0.991	0.245	1	0.621
<i>Cropland</i>												
<i>nifH</i>	28.573	2	< 0.01**	0.459	2	0.795	6.132	2	0.047*	8.039	2	0.018*
AOA	30.130	2	< 0.01**	4.250	2	0.119	5.797	2	0.055	10.706	2	< 0.01**
AOB	17.839	2	< 0.01**	3.397	2	0.183	8.767	2	0.013*	1.947	2	0.378
<i>nirK</i>	22.054	2	< 0.01**	2.299	2	0.317	10.208	2	< 0.01**	0.615	2	0.735
<i>nirS</i>	8.424	2	0.015*	0.515	2	0.773	2.665	2	0.264	3.048	2	0.218
<i>nosZ</i>	19.103	2	< 0.01**	7.186	2	0.028*	1.160	2	0.560	8.661	2	0.013*

Bold font indicates significant heterogeneity **, ($P < 0.01$; *, $P < 0.05$). NA means there were not enough valid groups for heterogeneity analysis

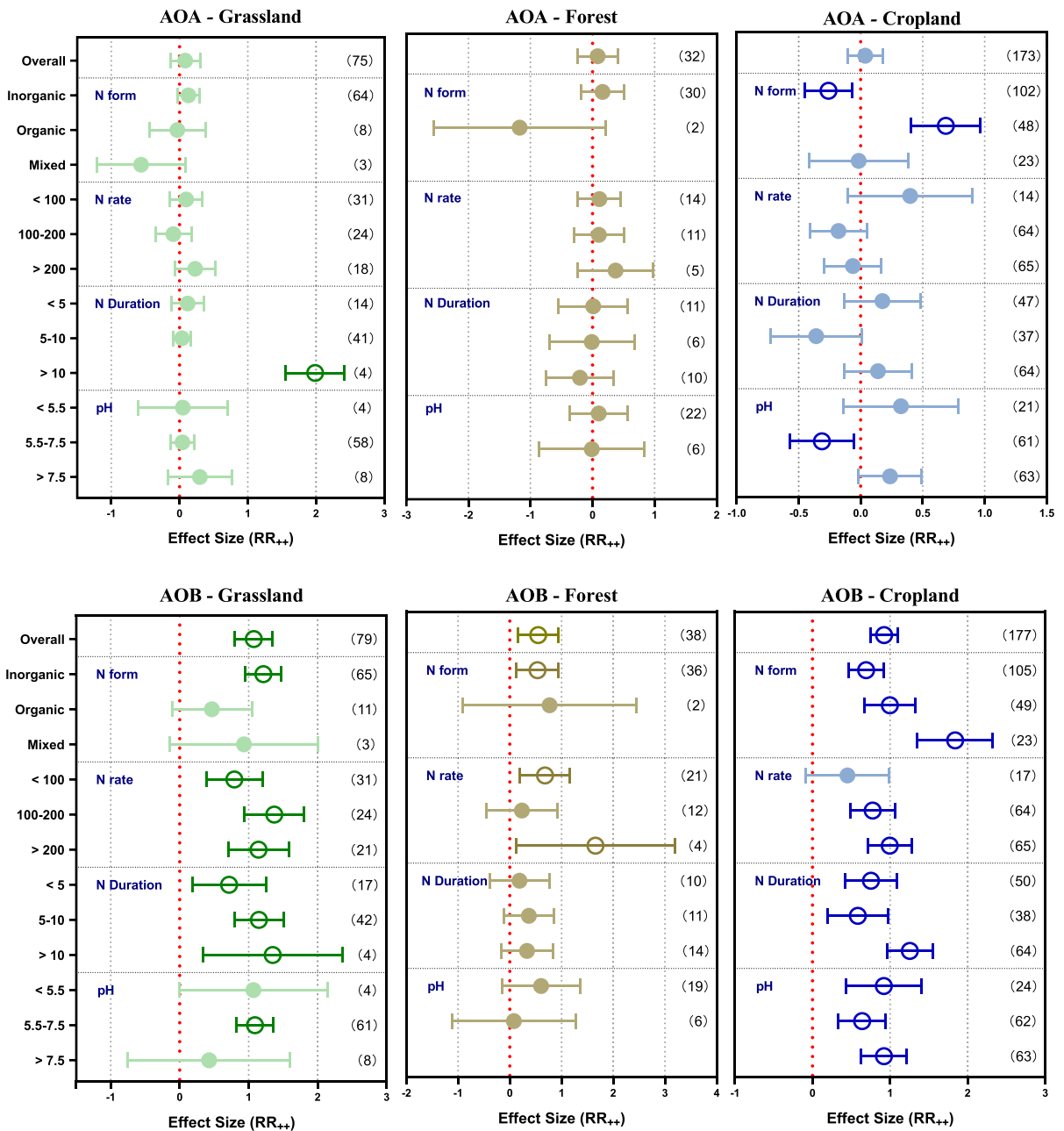
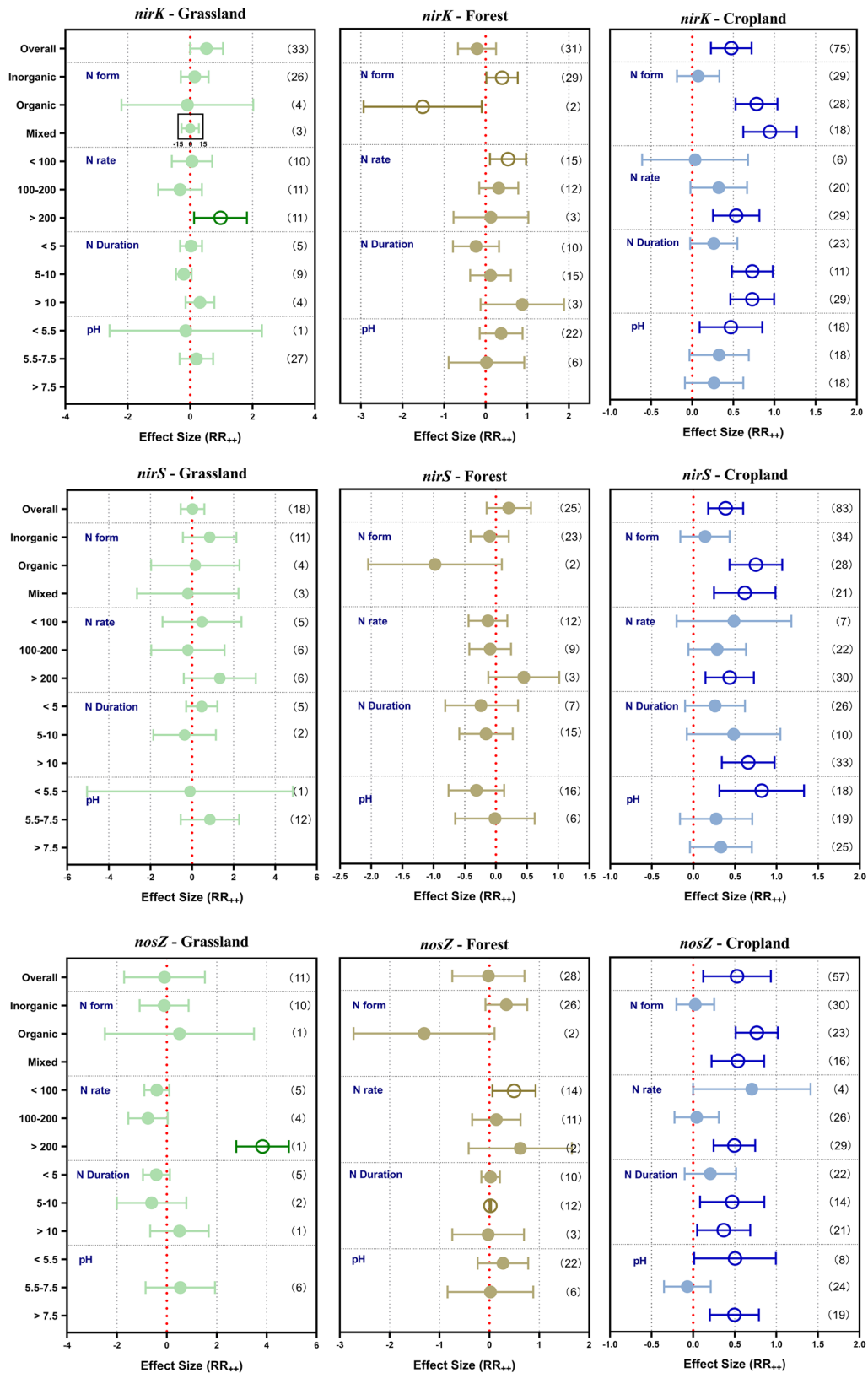


Fig. 3 The effect size of the *amoA* nitrification genes (AOA and AOB) to N additions in different ecosystems. Error bars indicate the 95% confidence interval. The results with signifi-

cant responses are indicated by hollow dots. The numbers in brackets indicate the number of studies included for each variable. ‘Overall’ indicates the abundance of each functional gene

and increased significantly under organic N applications and mixed N applications. The influences on the *nosZ* abundance varied among the ecosystems. The *nosZ* abundance was significantly affected by

the N application rate in grasslands, by the N form in forests, and by the N form, N rate, and pH in croplands.



◀**Fig. 4** The effect size of N additions on the denitrification genes (*nirK*, *nirS*, and *nosZ*) in different ecosystems. The error bars indicate the 95% confidence intervals. The results with significant responses are indicated by hollow dots. The numbers in brackets indicate the number of studies included in each variable. ‘Overall’ indicates the abundance of each functional gene

Correlations between climate and soil properties and N-cycling functional genes

We examined the effects of climate factors (MAT and MAP) and soil properties on the genes (Table 2). The *nifH* and *nosZ* were closely correlated to the climate variables in grasslands and croplands. AOA was closely related to N fractions in grasslands, while AOA and AOB were both strongly related to the pH in croplands. In addition, NO_3^- was important for the denitrifiers in croplands. Overall, there were strong relationships between TN and SOC and most genes in croplands.

The results from the model-averaged relative importance showed that different variables were important for the gene abundances in the different ecosystems (Fig. 5). There was no clear pattern for grasslands, with only a few important variables for a limited number of functional genes. In forests, the SOC was important for all the functional genes except *nosZ*. The cropland showed most effects from the N applications, with numerous important variables. SOC was important for all the functional genes, and pH was important for AOA, AOB, *nirS*, and *nosZ*. MAT, NO_3^- , and TN were important for *nifH*, AOA, and AOB.

We also examined the correlations between the effect sizes of the functional genes in the different ecosystems (Table S3). We found that, in grasslands, there were no significant correlations between the nitrification genes and denitrification genes, while *nifH* was significantly correlated with the nitrification genes. The nitrification genes were significantly correlated with denitrification genes in forests and croplands. The denitrification genes were positively correlated with each other in the three ecosystems.

Discussion

Effect of climate factors on the responses of N-cycling functional genes to N additions

We found that the responses of the *nifH* and *nosZ* genes were significantly and positively correlated

with the MAT and MAP in grasslands and croplands. These strong relationships suggest they were sensitive to changes in the external environment and may reflect the important role of MAT and MAP in controlling the exchange of N gases, namely NH_3 and N_2O . Chen et al. (2020) found that the response of the microbial activities to warming was minimal, and was closely related to the soil humidity. Homyak et al. (2017) found that the soil microbial biomass increased under small decreases in precipitation but decreased under large reductions in precipitation, with the changes related to the NH_4^+ concentrations. They also suggested that an ecosystem receiving N applications could lose N via a hydrological pathway when the precipitation subsequently increased, which would then reduce the production of N_2O . Thus, microorganisms are sensitive to changes in temperature and humidity in the environment, which will affect their gene expression (Cantarel et al. 2012; Klarenberg et al. 2021). MAT and MAP must be considered as predictors of functional genes because of their impact on the microbial activity and composition (Hu et al. 2020; Tang et al. 2019; Verma and Sagar 2020).

While the nitrification and denitrification genes and the climate factors were not closely related, the relative importance analysis indicated that temperature was important for the nitrification genes in croplands. Zhang et al. (2022) found that increases in temperature had a minimal effect on functional genes associated with N cycling in soil, reflecting the insignificant change on the plant biomass. However, warming and N additions combined caused noticeable increases in the abundances of *amoA*. Even if a temperature change causes a significant response in the soil substrate, the biological characteristics of AOA and AOB in autotrophic organisms could mean that their gene expression abundance does not change or even decreases (Kowalchuk and Stephen 2001; Tang et al. 2019). It could affect subsequent denitrification processes and the N_2O production (Cantarel et al. 2012; Ouyang et al. 2018).

Effect of N additions on the responses of the N-cycling functional genes

Nitrogenase, dominated by *nifH*, fixes N_2 to NH_4^+ , which is then followed by nitrification. However, this is not the only source of NH_4^+ . The soil NH_4^+

Table 2 Correlation analysis between climate and soil properties and functional genes in different ecosystems under N additions

Variable	<i>nifH</i>		AOA		AOB		<i>nirK</i>		<i>nirS</i>		<i>nosZ</i>	
	<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²	<i>P</i>	<i>R</i> ²
<i>Grassland</i>												
MAT	< 0.01**	0.516	0.074	0.059	0.184	0.033	0.429	0.032	0.308	0.205	0.592	0.051
MAP	< 0.01**	0.707	0.053	0.072	0.609	0.005	0.091	0.136	0.321	0.196	0.036*	0.546
pH	0.431	0.057	0.085	0.057	0.101	0.052	0.051	0.178	< 0.01**	0.769	0.020*	0.694
NH ₄ ⁺	0.262	0.113	< 0.01**	0.248	0.040*	0.093	0.802	0.003	0.071	0.599	0.234	0.269
NO ₃ ⁻	0.406	0.064	< 0.01**	0.310	0.041*	0.088	< 0.01**	0.529	0.057	0.549	< 0.01**	0.816
SOC	0.331	0.135	0.14	0.160	0.790	0.006	0.032*	0.308	0.939	0.004	0.360	0.410
TN	0.664	0.018	< 0.01**	0.625	0.944	0.000	0.994	0.000	0.399	0.362	0.094	0.661
<i>Forest</i>												
MAT	0.388	0.050	0.575	0.013	0.394	0.030	0.809	0.002	0.544	0.018	0.392	0.031
MAP	0.578	0.021	0.806	0.003	0.107	0.105	0.524	0.015	0.726	0.006	0.281	0.048
pH	0.518	0.028	0.510	0.034	0.132	0.136	0.045*	0.170	0.422	0.041	0.123	0.121
NH ₄ ⁺	0.615	0.017	0.782	0.006	< 0.01**	0.631	0.195	0.072	0.965	0.000	< 0.01**	0.355
NO ₃ ⁻	0.060	0.339	0.979	0.000	0.298	0.107	0.771	0.005	0.913	0.001	0.470	0.038
SOC	0.918	0.001	0.988	0.000	0.213	0.151	0.719	0.008	0.315	0.101	0.309	0.065
TN	0.041*	0.600	0.867	0.008	0.522	0.061	0.379	0.060	0.739	0.017	0.020*	0.434
<i>Cropland</i>												
MAT	< 0.01**	0.143	0.075	0.026	0.393	0.006	0.226	0.031	0.964	0.000	< 0.01**	0.144
MAP	0.065	0.045	0.153	0.015	0.255	0.009	0.202	0.030	0.064	0.056	0.034*	0.083
pH	< 0.01**	0.258	< 0.01**	0.242	< 0.01**	0.079	0.845	0.001	0.036*	0.096	0.773	0.002
NH ₄ ⁺	0.278	0.016	0.271	0.011	0.336	0.008	0.153	0.050	0.250	0.028	0.403	0.016
NO ₃ ⁻	0.143	0.029	0.402	0.006	0.727	0.001	< 0.01**	0.331	< 0.01**	0.169	< 0.01**	0.303
SOC	0.555	0.010	< 0.01**	0.167	< 0.01**	0.119	< 0.01**	0.384	0.042*	0.127	< 0.01**	0.473
TN	< 0.01**	0.223	< 0.01**	0.184	< 0.01**	0.133	< 0.01**	0.415	< 0.01**	0.179	< 0.01**	0.470

a. Mean annual temperature (MAT) and mean annual precipitation (MAP) were used in the logarithmically transformed data, Ln (MAT + 5 °C) and Ln (MAP mm), respectively (Li et al. 2020)

b. The size of the effect (*RR*₊₊) of the soil properties (pH, NH₄⁺, NO₃⁻, SOC, and TN) and the functional genes were used for the correlation analysis

c. Bold font indicates significant correlation coefficients (**, *P* < 0.01; *, *P* < 0.05). NA means there were insufficient valid groups for analysis

may increase when inorganic NH₄⁺-N is added from an external source or through mineralization of organic N by microorganisms (Nelson et al. 2016). This may help to explain why the *nifH* abundance does not change, and may even decrease, when inorganic NH₄⁺-N is applied to forests.

During nitrification, we found that the AOA abundance did not respond significantly to N additions in these ecosystems. This differs from the results reported by other researchers (Carey et al. 2016; Ouyang et al. 2018) and could reflect the small number of observations included in the other studies (Carey

et al. 2016; Ouyang et al. 2018). Furthermore, the ammonia monooxygenase that is encoded by AOA reaches saturation at a low NH₃ concentration. Studies have shown that, under low N applications, AOA dominates the nitrification process but, when the N application rate is high, the soil microbial activity decreases because of acidification (Di et al. 2009; Ouyang et al. 2018), and so the response of AOA to N additions is not significant (Prosser and Nicol 2012). The model-averaged relative importance also showed that the soil pH was an important control on the *amoA* abundance in croplands. Thus, AOB are more

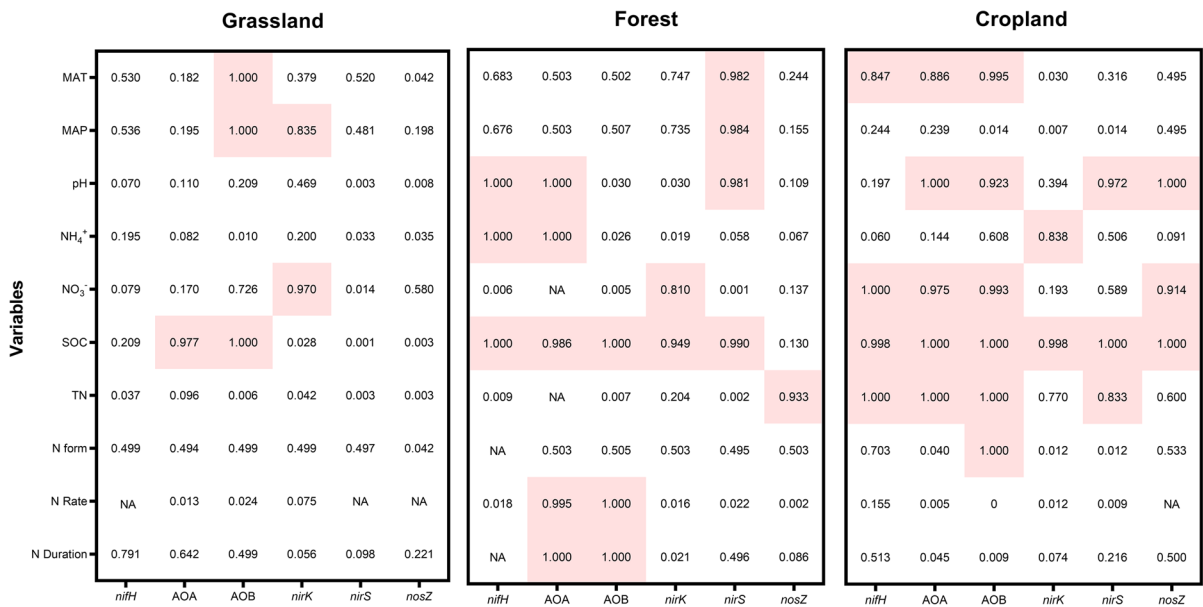


Fig. 5 The model-averaged relative importance of the predictors of N additions on different N-cycling functional genes in different ecosystems. The cut-off between important (red squares) and nonessential predictors (white squares) was 0.8.

adaptable than AOA and their response increases as the N contents increase (Prosser and Nicol 2012; Wang et al. 2016).

AOB were most abundant in grasslands, which had the highest inorganic N inputs and longest N application duration of the three ecosystems. This result is consistent with the physiological characteristics and NH₄⁺ oxidation pathways of AOB (Hu and He 2018; Ouyang et al. 2017; Prosser and Nicol 2012). The response of AOB reached a minimum in forests, suggesting that the N fixation rate was suppressed in forests (Dynarski and Houlton 2017). The increase in the AOB abundance was less in croplands (+151.86%) than in grasslands. Croplands receive more organic N than grasslands, and the complexity of the organic matter has little effect on nutrient decomposition and use of nutrients by microorganisms (Zhao et al. 2016). As a result, inorganic N has more effect on AOB than AOA at the same application rate and duration because AOB has a stronger affinity for NH₃ than AOA (Carey et al. 2016; Hu and He 2018; Ouyang et al. 2018).

The results show that the three denitrification genes were promoted most when organic N was added, with the promotion closely related to the nutrient

The importance was based on the sum of the Akaike weights derived from model selection using the corrected Akaike’s Information Criteria

content, and that SOC was an important driver of the responses of *nirK*, *nirS*, and *nosZ* to N additions (Fig. 5). Zhang et al. (2021) found that organic N had stronger effects on denitrifier abundances than inorganic N. Abdalla et al. (2019) reported that, as organic N applications to cropland under rotation increased, the NO₃⁻ leaching decreased and SOC retention improved. Thus, when supplied in large quantities, SOC and TN can promote the growth and physiological activities of soil microorganisms, especially heterotrophic denitrifying bacteria, causing the denitrification rate to increase (Morley and Baggs 2010) and enhancing the production of N₂O. Other studies reported similar results (Kallenbach and Grandy 2011; Kramer et al. 2006; Ouyang et al. 2018). Studies have shown that, as the rate and duration of the N applications increase, the denitrification rate and the production of N₂O will also change (Aronson and Allison 2012; Bai et al. 2014).

How soil properties affect the response of N-cycling functional genes to N additions

The soil pH has a strong influence on the microbial composition and gene abundance (Lauber et al.

2009). Functional communities each have unique optimum pH ranges and, for example, when the soil pH is greater than 5, the soil microbial biomass may increase significantly under N additions. External N applications have no effect on severely acidified soil (Geisseler and Scow 2014; Lauber et al. 2009; Ouyang et al. 2018). For example, unlike the grassland and cropland study sites, 84% of the soil in forest study sites examined in this analysis were already relatively acidic and had pH values between 3.7 and 5 before N applications commenced (Supplementary Dataset). Previous studies have reported that forest soils are frequently acidic (Friedland and Miller 1999). Researchers showed that, in forests, the proportion of AOA was higher than that of AOB, and that AOA preferred environments that had lower NH_3 contents and soil pH values (Assémien et al. 2017; Prosser and Nicol 2012; Srikanthasamy et al. 2018). When N is applied, the available N increases, which promotes the release of H^+ during litter decomposition and nitrification (Rao et al. 2009). Thus, the metabolism of microbes is suppressed in acidic soil. When analysing the roles of AOB and AOA in an acidified soil, Song et al. (2016) reported that AOA rather than AOB were responsible for nitrification in the acidic soil.

SOC and TN also influenced the gene abundance. Soil fertility in croplands can be maintained through crop rotation or planting ground cover during the slack season (Smith et al. 2008; Tilman et al. 2002; Zak et al. 2003). N applications could promote inputs of C and other exudates by stimulating the plant productivity and litter mass (Abdalla et al. 2019; Acosta-Martínez et al. 2007; Deng et al. 2020; Dodor and Ali Tabatabai 2005; Schmidt et al. 2011). A high C environment promotes the dissimilation and reduction of NO_3^- to NH_4^+ , which is then easily used by plants (Schmidt et al. 2011; Zhu et al. 2014). As the SOC and TN increase, the supply of NH_4^+ comes from directly improving the physiological metabolism of microbes and accelerating the decomposition of organic matter rather than through N_2 fixation (Cannavo et al. 2004; Cao et al. 2021). Therefore, as the SOC and TN increased, the *nifH* abundance either did not change or decreased. The nitrification functional genes, AOA and AOB, are also regulated by SOC and TN. The mineralization rate and soil organic matter are higher in forests than grasslands, which may have benefits for

nitrifier growth (Srikanthasamy et al. 2018). However, the microorganism activity and AOB abundance in forests may be low, because of the low inputs of organic fertiliser, and therefore SOC and TN (Cavicchioli et al. 2019). The microorganisms involved in denitrification are usually heterotrophic, and so compete for SOC (Tiedje 1988), which means that the denitrification genes are very sensitive to changes in SOC (Levy-Booth et al. 2014). Our analysis showed that SOC, which is important for plant productivity and microbial respiration (Fernández-Martínez et al. 2014), did not change significantly in grassland and forest ecosystems (Fig. 1), which helps to explain the lack of significant change in the denitrification gene abundance.

In general, the climate factors and N together influence the soil physical and chemical properties, and so affect the abundance of the various functional genes and their contributions to N cycling. Because of the unique properties of soil and microbial communities in different ecosystems, the degree of change in the soil properties and in the abundances and responses of the functional genes will differ. We summarized the results from our analysis and developed a conceptual model of how N additions and climate influence soil properties and N-cycling functional genes (Fig. 6). We believe our model will be mainly valid for temperate soils based on the sources of our data (Fig. S1). Some of the soil properties, such as NO_3^- , pH, and NH_4^+ , and functional genes (AOB) changed considerably under N additions across all ecosystem types. The abundance of *nifH* decreased significantly (by 32.79%) in forests and the abundances of *nirK*, *nirS*, and *nosZ* increased significantly in croplands. The results demonstrate the role of climate and, in future, we suggest that MAT and MAP should be considered as predictors of functional genes because of their impact on the microbial activity, and the composition and concentrations of the reaction substrate. We advise careful nutrient budgeting and monitoring of the pH and NH_4^+ , to avoid over applications of N, that drive increases in emissions, and consequent acidification of the soil and pollution of nearby waterways by NO_3^- .

Conclusion

This study applied meta-analysis techniques to data from 119 articles, to identify how N additions caused

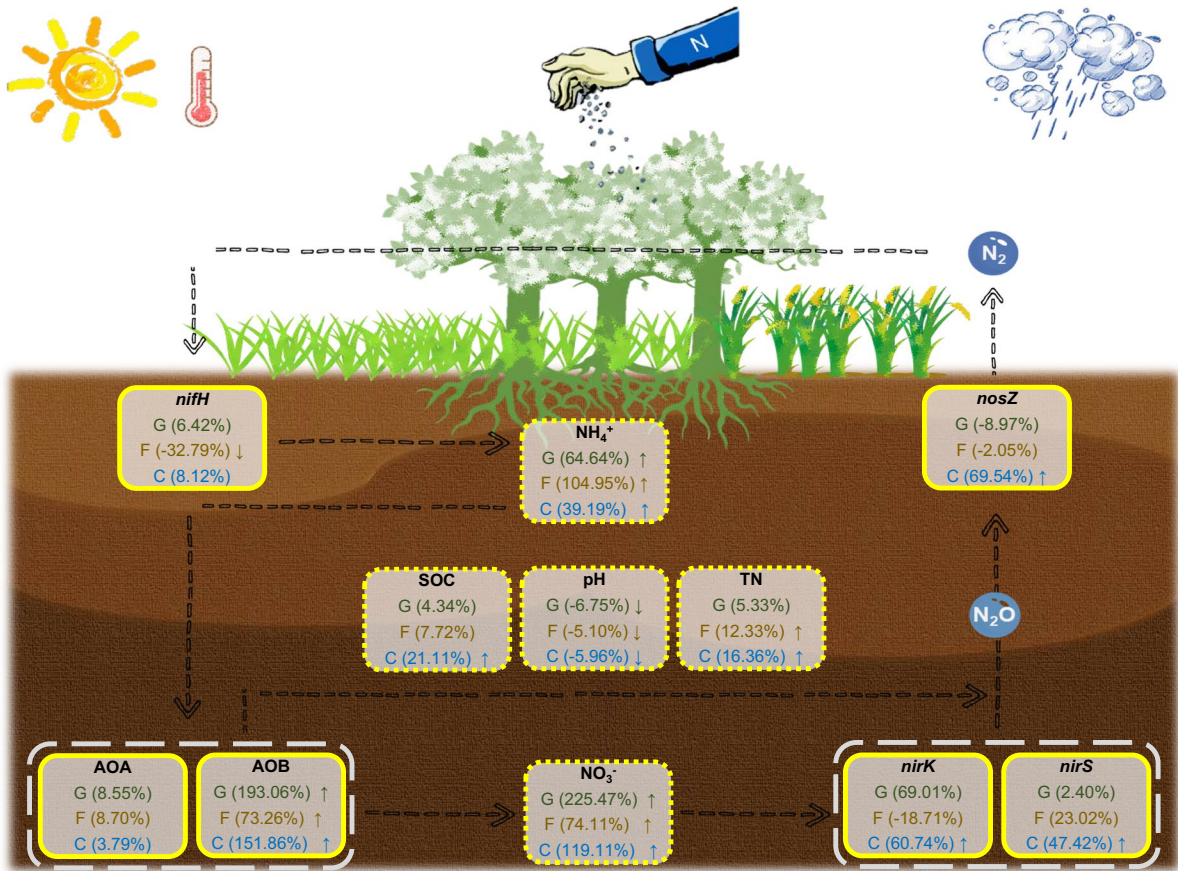


Fig. 6 Conceptual model of the mechanisms driving the changes in the N-cycling functional genes in different ecosystem soils under N applications. G means grassland, F means forest, and C means cropland. The number with an arrow represents a significant change

changes in the N-cycling functional genes in three different ecosystems. We found that the responses of the N-cycling functional genes to N additions varied among the ecosystems. Under N applications, the AOA abundance did not change significantly in the three ecosystems, the AOB abundance in grasslands increased by 193.06%, the *nifH* and AOB in forests changed significantly, and the AOB and denitrification gene (*nirK*, *nirS*, and *nosZ*) abundances in croplands increased significantly. The N-cycling genes were influenced by different variables among the ecosystems with most effects in the croplands. There were no strong overall patterns for the N-cycling genes in grasslands. The N-cycling genes in forests were mainly influenced by SOC, but in croplands, were influenced by the MAT, pH, NO_3^- , SOC, and TN. Through changing the reaction substrate, and affecting the microbial activity, the climate, N

additions, and soil properties impacted the expression abundance of the functional genes. Quantifying the differences in the responses of the N-cycling gene abundances to N additions across three terrestrial ecosystems in this way can produce useful information about gene-based models of N cycling in soil, especially the populations and activities of microbial functional communities.

Acknowledgements We would like to thank the reviewers and editor for proofreading and providing helpful suggestions on the manuscript. Sincere appreciation to the scientists whose research studies were included in this meta-analysis. This study was supported by the Second Tibetan Plateau Scientific Expedition and Research Program (STEP) [Grant No. 2019QZKK0608] and the National Natural Science Foundation of China [Grant No. 31770519].

Authors' contributions All the authors contributed to the study conception and design. Yinghui Liu and Jingyi Dong

conceived the ideas and designed the methodology; Jingyi Dong, Jiaqi Zhang, and Haichao Jing collected the data; Jingyi Dong and Jiaqi Zhang analysed the data, and Jingyi Dong wrote the manuscript.

Data availability All the data presented in this manuscript are available in the supporting information.

Declarations

Conflict of interest There is no conflict of interest.

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