Title

Alterations in soil pH emerge as a key driver of the impact of global change on soil microbial nitrogen cycling: Evidence from a global meta-analysis

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Authors

Zhong, Yangquanwei

Yan, Weiming

Canisares, Lucas P

et al.

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Title page

Title: Alterations of soil pH emerges as a key driver of the impact of global change experiments on soil microbial abundance and nitrogen cycling pathways

Running title: Responses of N cycling to global change

Authors' names: Yangquanwei Zhong [1], Weiming Yan [2*], Lucas P. Canisares [3,4], Eoin L. Brodie [4,5 *]

Author affiliations:

1 School of Ecology and Environment, Northwestern Polytechnical University, Xi'an, 710072, P.R. China

2 State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China

3 Soils and Environmental Resources Center, Agronomic Institute of Campinas, Campinas, Brazil

4 Ecology Department, Climate and Ecosystem Sciences Division, Earth and Environmental Sciences Area, Lawrence Berkeley National Laboratory, Berkeley, CA, United States

5 Department of Environmental Science, Policy and Management, University of California, Berkeley, Berkeley, CA, United States

***Corresponding author:**

Weiming Yan, E-mail: weimingyan@lbl.gov

Address: Xinong Rd. 26, Institute of Soil and Water Conservation, Yangling, Shaanxi 712100, PR China

Eoin L. Brodie, E-mail: elbrodie@lbl.gov

Address: One Cyclotron Road, Berkeley, California 94720, United States

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Abstract

The cycling of nitrogen (N) is critical to the productivity of terrestrial ecosystems and

to their response to global change (Terrer et al., 2021). However, the impact of global change factors (GCFs) on the microbial mediators of critical N cycling pathways is highly variable and has yet to be synthesized. Here, we synthesized 8322 paired observations of soil microorganisms related to N cycling from experiments around the world where GCFs (elevated CO_2, warming, water availability, nutrient addition) were manipulated. We found that the abundances of archaea, bacteria, and most N cycling genes are relatively resistant to elevated CO_2 concentration, experimental warming and water reduction, while water addition strongly stimulated most genes related to N cycling. N addition and the combination of N addition with other GCFs significantly increased the abundance of ammonia oxidizer bacteria (amoA-AOB), which also showed different responses to N addition in upland and lowland soils. Random forest analysis indicated that under steady-state conditions (controls in these experiments) the most important variables driving global soil bacterial, archaeal and N-cycling gene abundances varied in terms of the contribution of climatic, edaphic and spatial variables. However, upon experimental manipulation, the induced change in soil pH was the most important factor driving changes in soil microbial and N-cycling pathways under GCFs manipulation. Importantly, the changes in ammonia-oxidizing archaea (amoA-AOA) and bacteria (amoA-AOB), as well as genes involved in denitrification (nirS and nosZ) were significantly correlated with the rates of their corresponding processes. While, changes in the abundance of genes related to denitrification were in some cases inconsistent with N transformation process rates under GCFs. We found that GCF-induced shifts in the potential nitrification rate (PNR) are well explained by changes in abundance of the amoA-AOB under GCFs. Together, our results imply that GCFs that induce changes in soil pH may have a profound impact on the terrestrial N cycle.

Introduction

Nitrogen (N) cycling is critical for the functioning of terrestrial ecosystems (Kuypers et al., 2018; Ouyang et al., 2018)) and plays an essential role in mediating N exchange

along the soil-plant-atmosphere continuum (Carey et al., 2016; Dai et al., 2020; Séneca et al., 2020). Due to anthropogenic activities, terrestrial ecosystems are currently undergoing irreversible global changes, such as global warming, rising carbon dioxide levels (eCO_2), changes in precipitation, nutrient enrichment, and combinations of these changes; they may alter plant growth, soil microclimates, soil substrate availability, and soil microbial communities and their functions, resulting in profound impacts on soil N cycling (Kuypers et al., 2018; Ouyang et al., 2018; Dai et al., 2020; Li et al., 2020a; Séneca et al., 2020). Thus, a comprehensive understanding of the effect of global change factors (GCFs, climate change and nutrient enrichment) on N cycling processes is essential for predicting future N cycling dynamics.

Soil N transformation processes are largely driven by microorganisms, including a diverse range of archaea and bacteria that regulate nitrification, denitrification and N fixation (Kuypers et al., 2018). Several functional gene markers are often used to study the abundance and composition of the microbial communities responsible for these N transformation processes. The amoA gene (encoding ammonia monooxygenase subunit A) is often used to quantify populations of ammonia-oxidizing archaea (amoA-AOA) and bacteria (amoA-AOB) (Rotthauwe et al., 1997; Pester et al., 2012; Séneca et al., 2020)); the nirK/nirS (encoding nitrite reductase) (Braker et al., 1998; Bárta et al., 2010) and nosZ genes (encoding nitrous oxide reductase) are used to quantify different groups of denitrifiers (Scala and Kerkhof, 1998; Juhanson et al., 2017); while the nifH gene (encoding nitrogenase reductase) is used to quantify N fixers (Zehr et al., 1998; Ouyang et al., 2018). Monitoring changes in the abundances of these key functional genes involved in soil N transformation represents an important approach to understand how N processes and their microbial catalysts vary across soils and with perturbation (Ouyang et al., 2018; Xiao et al., 2019).

The oxidation of ammonia to NO_2^- by amoA-AOA and amoA-AOB is considered to be the first and rate-limiting step of the nitrification process (Xiao et al., 2019). The ratio of amoA-AOA to amoA-AOB varies greatly in different soil ecosystems (Leininger et al., 2006; Yao et al., 2011; Yao et al., 2013). Some studies have observed significant increase in amoA-AOB over amoA-AOA in N-rich soils (Di et al., 2009; Jia and Conrad, 2009), while others have reported that amoA-AOA is the

main ammonia-oxidizing taxon in N-depleted and acidic soils because amoA-AOA are more adapted to oligotrophic environments (Erguder et al., 2009; Zhalnina et al., 2012; Zhang et al., 2012). The differences between amoA-AOB and amoA-AOA are mainly due to the different ammonia affinities, availability of C substrates, and optimal growth pH values of amoA-AOA and amoA-AOB (Prosser and Nicol, 2012; Kits et al., 2017), which may cause amoA-AOA and amoA-AOB to have different responses to GCFs (Hu et al., 2016; Zhang et al., 2017; Séneca et al., 2020). Denitrification is considered to be a four-step process and is primarily anaerobic. NirS and nirK are involved in the second step of denitrification, nosZ is responsible for the reduction of nitrous oxide, and different responses of these genes to GCFs are often observed (Petersen et al., 2012; Brenzinger et al., 2017). Soil temperature, moisture/O_2 availability, pH, carbon (C) and mineral co-factors are key regulators of the denitrifier activity; changes in one or more of these factors can have a large influence on both the denitrifier community activity and its diversity (Wallenstein et al., 2006). Some studies have observed that denitrifiers with the nirK gene were more sensitive to environmental changes (elevated temperature, soil moisture and CO_2 enrichment) than the nirS gene (Jung et al., 2011; Nadeau et al., 2019; Diao et al., 2020). In addition, the nifH gene is often used for the detection of N-fixing bacteria and archaea, which are also impacted by edaphic factors, such as soil texture and inorganic nitrogen (Riffkin et al., 1999; Poly et al., 2001).

Many individual studies have examined the response of N-cycling gene abundances to GCFs, but inconsistent results have often been observed (Ouyang et al., 2018; Li et al., 2020a; Séneca et al., 2020). For example, eCO_2 can promote both plant growth and photosynthesis (Dusenge et al., 2019), increase belowground C allocations, stimulate microbial growth and reduce soil available N, which may result in increased abundances of ammonia oxidizers and denitrifiers (Liu et al., 2015; Diao et al., 2020). However, increased C inputs can indirectly affect soil microbes by gradually resulting in limitations on other nutrients (particularly N), leading to a decrease in ammonia oxidizer and denitrifier abundance in response to eCO_2 (Horz et al., 2004; Regan et al., 2011; Sun et al., 2018). In addition, ammonia oxidizers, denitrifiers and N fixers have been reported to show no response to CO_2 enrichment (Long et al., 2012; Brenzinger et al., 2017; Zhang et al., 2017). In contrast to eCO_2,

warming and precipitation changes can directly affect microbial processes by changing the soil temperature, moisture and nutrient availability, and divergent responses of the abundances of amoA-AOA, AOB (Tourna et al., 2008; Delgado‐Baquerizo et al., 2014; Zhang et al., 2017; Séneca et al., 2020) and nirS, nirK and nosZ genes to warming and precipitation changes have been observed (Zhang et al., 2013; Zhang et al., 2017; Qiu et al., 2018), as well as for nifH genes (Yeager et al., 2012; Zhang et al., 2013; Carrell et al., 2019). In addition, climate from 50 years ago might have significant effect on current distributions of soil bacteria because it was much slower for soil properties adapting changes in climate (Ladau et al., 2018). However, whether climate change manipulation have influence on N-cycling gene abundances still have debate.

Nutrient addition also impacts N-cycling gene abundance by directly increasing soil nutrient availability or indirectly increasing plant growth and belowground C allocations and alteration of soil pH due to root uptake and microbial activity (Dong et al., 2015; Ning et al., 2015). However, excessive N addition reduces the allocation of photosynthetic C to the root and decreases the soil pH; thus, inconsistent responses of N-cycling gene abundance to nutrient addition have been reported (Nakaji et al., 2001; Berthrong et al., 2014; Ouyang et al., 2018; Tang et al., 2019).

The responses of soil bacteria and archaea and their N-cycling gene abundances to GCFs at the global scale remain uncertain and require further investigation. Several meta-analyses of N-cycling gene abundances have been conducted (Carey et al., 2016; Ouyang et al., 2018; Dai et al., 2020; Li et al., 2020a; Salazar et al., 2020), but these studies have focused mainly on the effect of a single manipulation of N addition or temperature or on only some of these N cycling genes. Therefore, a comprehensive global analysis of soil bacteria and archaea and their N-cycling gene abundances under GCFs and their combination can provide important information for understanding N processes and associated mechanisms under future global change.

Here, we conducted a meta-analysis based on 8,322 paired observations derived from 151 publications that reported the site-specific effects of climate change and nutrient addition on soil microbes, N-related functional genes, N cycling processes and soil physical-chemical properties. The objectives of this study were to investigate (1) the global patterns and responses of soil bacteria and archaea and key N-cycling

gene abundances to climate change and nutrient addition; (2) major factors that drive changes in soil bacteria and archaea and key N cycling genes; and (3) whether the changes in key N cycling genes impact N cycling processes (nitrification, denitrification, and fixation).

Materials and Methods

Data preparation

We searched the Web of Science and CNKI databases for peer-reviewed publications published until August 2020, with no restrictions on the publication year. The keywords were ["ammonia oxidizers" or "nitrifiers" or "denitrifiers" or "N_2 fixation" or "amoA gene" or "nirS gene" or "nirK gene" or "nosZ gene" or "nifH gene"] and "climate change" or "elevated CO_2" or "CO_2 enrichment" or "warming" or "elevated temperature" or "rising temperature" or "precipitation change" or "altered precipitation" or "increased precipitation" or "precipitation increment" or "drought" or "N application" or "N fertilization" or "N deposition" or "N addition" or "P addition" or "fertilizer application" or "nutrient management". To effectively represent the responses of soil microbes and N-cycling genes under natural conditions, the following criteria were used to avoid publication bias in data selection: 1) only field studies were selected, and laboratory incubation and pot studies were not included; 2) quantitative PCR was used to measure the abundance of the soil microbes and N-cycling genes; 3) the selected studies had at least two datasets (control and treatment) with the same experimental duration, and the mean, standard deviation/error and number of replicates in the control and treatment groups could be either directly extracted from the text, tables, or digitized graphs or calculated; and 4) measurements with different experimental durations within the same study were treated as individual observations. A total of 3905 paired measurements of climate change and nutrient addition studies were derived from 151 publications (Supplementary references) worldwide (**Fig. 1a**). In total, 8,322 paired measurements were derived from 151 publications, of which 3,905 observations were of soil microbial and N-cycling gene abundances, and 4,417 observations were of soil physical-chemical properties.

In addition to the soil microbial and N-cycling gene abundance metrics, the

datasets also included geographic and environmental variables, such as location (i.e., latitude and longitude), mean annual temperature (MAT), mean annual precipitation (MAP), ecosystem type (including upland and lowland), experimental forcing factors (i.e., magnitude of warming, eCO_2, percentage change in precipitation, fertilization rates of N, P or K), and the length of the manipulation experiment (**Table S1**). Any simultaneously available data on soil temperature, moisture, pH, soil organic carbon (SOC), dissolved organic C (DOC), total N, NH_4^+-N, NO_3^--N, microbial biomass C (MBC) and N (MBN) in the control and treatment groups were also collected (**Table S1**). Overall, the dataset covered broad variations in GCF manipulation type: climate, landscape position, magnitude of GCFs, and experimental duration, and it included six single-factor GCF experiments (i.e., eCO_2, warming, precipitation addition (PPT+), precipitation reduction (PPT-), and N (N) and P (P) addition) and ten combined factors (i.e., N × P, N × P × potassium (K) addition, warming × N, warming × PPT-, warming × PPT+, warming × eCO_2, N × PPT+, N × PPT-, N × K, and N × eCO_2) (**Supplementary Dataset**). For studies in which the data were presented graphically, Get–Data Graph Digitizer (ver. 2.20, Russian Federation) was used to digitize and extract the numerical data.

Data analysis

The effects of the GCFs on N-cycling genes and soil properties were calculated using the natural log response (lnR_i) for each case study using the following equation (Hedges et al., 1999):

$$lnR_i = ln(Xt_i/Xc_i) = lnXt_i - lnXc_i \quad (1),$$

where Xt_i and Xc_i are the means of each observation for the treatment and control groups, respectively. The corresponding sample variance was calculated as:

$$v = (S_t/Xt_i)^2/n_t + (S_c/Xc_i)^2/n_c \quad (2),$$

where n_t and n_c are the sample sizes of the treatment and control groups, respectively, and S_t and S_c are the standard deviations for the treatment and control groups, respectively. The following equation was used as the weighting factor (w_i) for each response ratio (RR):

$$w_i=1/v \quad (3).$$

In this study, the mean weighted RR of all observations for the treatment group was calculated from ln R using a categorical random effect model as follows:

$$\text{mean lnR} = \sum_{i=1}^{n} w_i R_i / \sum_{i=1}^{n} w_i \quad (4),$$

where n is the number of observations. In addition, the standard error of the mean lnR was estimated as follows:

$$\text{standard error (RR)} = \sqrt{\frac{1}{\sum_{i=1}^{n} w_i}} \quad (5).$$

The overall mean RR and its confidence interval (CI) for each grouping category were generated by mixed-effects models using the rma.mv function from the 'metafor' package. The treatment effects were considered nonsignificant if the 95% CI overlapped zero; otherwise, the treatment effect was considered significant. In addition, groups with less than two paired measurements were excluded from the study to meet the criteria for meta-analysis rigor.

Meta-analysis was performed using the R software package (version 4.1.1) (Team, 2020). The R package 'ggplot ()' was used to chart the kernel density estimates (a smoothed version of the histogram) of the greenhouse gas fluxes (**Fig. S1**). A restricted maximum likelihood mixed-effects model was used for the meta-regression in the OpenMEE software package to explore the effect of each categorical group (biome, MAT, MAP, duration, and N rate and form) on the variables. A random forest analysis using the R package 'rfPermute' was used to identify the relative importance of environmental variables in influencing the responses of soil microbe and N-cycling gene abundance under the GCFs. In addition, a model selection analysis using the R package 'glmulti' () was performed to calculate the relative importance of all predictors for the soil microbe and N-cycling gene abundances. 'glmulti' identifies the 'n' best models (the confidence set of models) among all possible models (the candidate set, as specified by the user). Models are fitted with the specified fitting function (default is glm) and are ranked with the specified information criterion (the default is the Akaike information criterion corrected for small sample sizes (AICc)). The best models are identified either through exhaustive screening of the candidates or with a genetic algorithm, which allows very large candidate sets to be addressed. The importance value for a particular predictor is equal to the sum of the weights/probabilities for the models in which the

variable appears. Therefore, a variable that appears in many models with high weights will receive a high importance value. In that sense, these values can be regarded as representing the overall support for each variable across all models in the candidate set. In addition, regression analysis between the RRs of soil microbial and N-cycling gene abundances and other variables was conducted.

Results

Abundances of archaea, bacteria and key N-cycling genes globally

Across the control treatments in all studies, bacterial abundances were higher than archaeal abundances, and amoA-AOA abundances were higher than those of amoA-AOB in both upland and lowland ecosystems (**Fig. 1b**). However, the abundances of the nirS, nosZ and nifH genes were significantly higher in lowlands than in uplands. Among the different biomes, archaeal and bacterial abundances were higher in grasslands than in forests and croplands, and forests showed the lowest abundances of archaea, bacteria, amoA-AOA, amoA-AOB, and nirK (**Fig. S2**). The abundances of bacteria, archaea and N-cycling genes were correlated with climate (MAT and MAP), spatial (latitude and longitude), and soil physical-chemical properties (SOC, pH, NH_4^+, NO_3^- and TN) (**Table S2**). The random forest analysis showed that these environmental factors were differentially important to the bacterial, archaeal and N-cycling gene abundances ($p<0.001$) (**Fig. 1**).

Effects of GCFs on archaea, bacteria and key N-cycling gene abundances

In this study, we found that GCFs did not always cause consistent changes in the abundances of archaea, bacteria and key N-cycling genes (**Fig. 2**). For example, eCO_2 and warming in general showed no consistent effect on archaea, bacteria, and N-cycling gene abundances, although warming consistently decreased the abundance of nirK genes. However, PPT+ significantly increased the abundances of bacterial, amoA-AOA, nirS, nirK and nosZ genes, while PPT- increased only the nirK gene abundance. N addition significantly decreased the abundances of archaea and nosZ, nifH, hao, norB and napA genes and increased those of fungi and amoA-AOB genes (**Fig. 2 and S3**). P addition increased the abundance of nosZ genes, and the combination of N and P addition decreased the abundance of nifH and amoA-AOA genes but increased that of the amoA-AOB gene, whereas the combination of N, P

and K addition increased the abundances of bacteria and amoA-AOB, nirS, nirK and nosZ genes. Moreover, we found that amoA-AOB showed a positive response to GCFs in combination with N addition, and nirK genes were more sensitive to GCFs in combination with precipitation changes (**Fig. S4**).

The effect sizes for bacterial and N-cycling gene abundances differed between different habitats (upland and lowland) and among different biomes (croplands, forests, and grasslands) (**Figs. 3 and S5**). N-cycling genes in uplands and lowlands showed opposite responses to N addition, but similar trends of N-cycling genes were observed under combined N, P and K addition. The abundances of amoA-AOB and nirS genes showed a positive response to warming in lowlands. In addition, the experimental duration significantly affected the abundances of amoA-AOA, amoA-AOB, nirK and nosZ genes under N addition (**Table S3**), while N form, climate, and habitat also impacted the abundances of N-cycling genes (**Tables S3 and S4**). In addition, the magnitude and duration of the manipulation of GCFs also affected the abundances of N-cycling genes (**Table S5**).

Factors driving the responses of archaea, bacteria and key N-cycling genes to GCFs

Edaphic factors showed different responses to the GCFs (**Table S6**), and climate, spatial and edaphic environmental factors showed different correlations with the changes in bacterial, archaeal and N-cycling gene abundances (**Table S7**). The model selection analysis indicated that changes in edaphic factors induced by GCFs were the main predictors of bacterial, archaeal and N-cycling gene abundances (importance>0.8) **(Fig. S6**). When averaging the importance of these predictors, we found that changes in soil pH induced by GCFs were the best predictor of bacterial, archaeal and N-cycling gene abundance changes **(Fig. 4)**. Further, weighted random forest analysis also showed that changes in soil pH were the most important moderators of the bacterial, archaeal and N-cycling gene abundance changes under GCFs (**Fig. S6a-g**); the only exception was the nifH gene, for which MAT was the most important moderator (**Fig. S6i**). Furthermore, the RRs of the bacterial, archaeal and N-cycling gene abundances ($P < 0.01$) significantly increased as the soil pH increased (**Fig. 5 a-c and e-h**); although, the RR of amoA-AOB showed a weak opposing response to changes in soil pH (**Fig. 5d**).

Relationships between changes in key N-cycling genes and their corresponding N processes

In this study, GCFs were also shown to impact N transformation processes; for example, warming promoted N mineralization and N_2O emissions, while nutrient addition had a significant effect on most N transformation processes (**Fig. 6**). The RRs of the bacterial, archaeal and key N-cycling gene abundances were highly correlated with each other (**Table S4**). In addition, different relationships between changes in bacteria, archaea and N-cycling genes and their corresponding N-processes were observed (**Fig. 6**). For example, the RR of the amoA-AOA and amoA-AOB genes both showed a positive relationship with the RR of the potential nitrification rate (PNR) (**Fig. 7a and b**). An increase in the nirK gene abundances also significantly correlated with the RR denitrification enzyme activity (DEA) (**Fig. 7c**). However, significant relationships between effect sizes under GCFs were only observed for amoA-AOB and PNR (**Fig. 7g**).

Discussion

To our knowledge, this meta-analysis is the first to provide a comprehensive evaluation of soil microbial and N-cycling gene abundances and their response to a variety of GCFs. Compared with previous meta-analyses focusing only on some N-cycling genes under N addition (Carey et al., 2016; Ouyang et al., 2018) or elevated temperatures (Dai et al., 2020), this study provides a more extensive database of soil bacterial, archaeal and N-cycling gene abundances under a variety of GCFs and links them to environmental factors and N transformation processes with synchronous datasets. Our results showed the varied response of soil archaea, bacteria and N-cycling genes to GCFs and highlighted that changes in soil pH induced by GCFs are frequently the main driver. In addition, functional gene responses to GCFs were generally correlated with their corresponding process rates, however denitrification gene abundances did not necessarily reflect N transformation process rates under the different GCFs.

Differential responses of soil archaea, bacteria and key N-cycling gene abundances to GCFs

In steady-state soils (controls without GCF treatments), although a higher abundance of bacteria than archaea was observed, the abundance of amoA-AOA was consistently higher than that of amoA-AOB (**Fig. 1b**); this is consistent with most previous studies in various regional environments (Leininger et al., 2006; Adair and Schwartz, 2008; Yao et al., 2011; Yao et al., 2013). In addition, environmental factors, including climate, spatial and edaphic factors and habitat, have differential importance to soil archaeal, bacterial and N-cycling gene abundances (**Fig. 1c**), consistent with previous studies (Bru et al., 2011; Gubry-Rangin et al., 2011; Yao et al., 2013; Wei et al., 2020).

Across all studies, differential responses of soil bacteria, archaea and N-cycling gene abundances to GCFs were observed. In general, increasing CO_2 is thought to promote plant growth and increase C inputs to the soil, which may indirectly result in soil substrate (NH_4^+ and NO_3^-) depletion for nitrifiers and denitrifiers (Fierer, 2017). However, we found that archaeal and bacterial abundances and nitrifier, denitrifier and N-fixer functional gene abundances were relatively insensitive to eCO_2 (**Fig. 2a**). This may have been mainly due to the limited impact on soil pH, DOC, NH_4^+ and NO_3^- under eCO_2 (**Table S3**), and previous studies have reported that changes in soil properties such as pH and N substrate, may have a stringer impact than eCO_2 on soil microbes and N-cycling genes (Long et al., 2012; Brenzinger et al., 2017; Zhang et al., 2017).

In warming studies, we found that soil archaea, bacteria, amoA genes, nirS, nosZ and nifH genes were less sensitive to elevated temperature (**Fig. 2b**). Warming reduced soil moisture and NH_4^+ availability, possibly imposing a further constraint on stimulatory responses (**Table S6**), consistent with previous studies (Zhang et al., 2017; Séneca et al., 2020). However, warming consistently decreased the nirK gene abundance among denitrifiers (**Fig. 2b**), as nirK communities are negatively affected by increased temperature (Jung et al., 2011; Xu et al., 2016; Li et al., 2020b). In addition, amoA-AOB and nirS genes showed positive responses to warming in lowlands, which might relate to the higher NH_4^+ in lowlands (131.4 mg kg^{-1}) than upland (11.2 mg kg^{-1}).

Although water addition had no consistent effect on SOC, DOC, NH_4^+ or NO_3^-, it significantly increased the bacterial, amoA-AOA, nirK, nirS and nosZ gene

abundances; this could be explained by the consistent increase in soil moisture and pH, as N-cycling genes showed a positive correlation with soil pH (**Table S4**) (Ouyang et al., 2018; Xiao et al., 2019). However, although water reduction decreased soil moisture, it had no consistent effect on the abundance of bacteria, nitrifiers or denitrifiers, except that of the nirK gene. This may have been because the soil pH, SOC, DOC, NH_4^+ and NO_3^- (**Table S6,** $P>0.05$) were unchanged, possibly because most studies were conducted for less than three years and we found that response ratios were very sensitive to study duration. These results indicate that effects of water reduction on substrate availability rather than soil moisture play a more important role in influencing soil microbial and N-cycling genes (Fuchslueger et al., 2014; Chen et al., 2017). Interestingly, water addition and reduction both promoted increased nirK gene abundance, and the RR of the nirK gene showed positive and negative relationships with the magnitude of annual precipitation change under water addition and reduction, respectively (**Table S5; Fig. S7**). These results suggest that microorganisms that have the nirK gene exhibit higher soil moisture sensitivity. This unexpected result might be related to the relatively high MAP in the water reduction studies (a mean MAP of 817 mm across all water reduction experiments); a previous study found that nirK abundance showed a nonlinear relationship with soil moisture and that water reduction may not cause water stress in nirK-harboring microorganisms (Chen et al., 2019).

Different effects of nutrient addition on soil archaeal, bacterial and N-cycling gene abundances were observed (**Fig. 2 e-h**). N addition significantly increased the abundance of only amoA-AOB and not that of amoA-AOA (**Fig. 2 e**), and amoA-AOB significantly increased under GCFs combined with N addition (**Fig. 2 g and h; Fig. S4**). These results indicate that amoA-AOB are more responsive than amoA-AOA to N addition, which is consistent with previous meta-analyses that reported a higher sensitivity of amoA-AOB than amoA-AOA to N application (Carey et al., 2016; Ouyang et al., 2018). This overall finding could be attributed to the different affinities for NH_3 and ammonia oxidation kinetics of amoA-AOA and amoA-AOB (Carey et al., 2016; Kozlowski et al., 2016; Ouyang et al., 2018); amoA-AOB has a higher maximum activity and half-saturation constant than amoA-AOA (Hatzenpichler, 2012; Ouyang et al., 2017). In addition, a decrease in soil

pH might also be an important factor driving niche differentiation between amoA-AOA and amoA-AOB (**Tables S6**) (Prosser and Nicol, 2012; Xiao et al., 2019). However, in this study, for amoA-AOA and amoA-AOB, respectively, we found smaller percentage change of 3% (362 observations) and 139% (380 observations), compared with previous results, i.e., 27% (98 observations) and 325% (117 observations) including laboratory and greenhouse studies (Carey et al., 2016) and 31% (98 observations) and 313% (106 observations) in only cropland studies (Ouyang et al., 2018).. In addition, Ouyang et al. (2018) found that N addition significantly increased the abundances of nirK, nirS and nosZ genes but had no effect on nifH gene abundance, whereas we observed significant reductions in nosZ and nifH genes abundances. This may have occurred because P and K fertilizers were often added in combination with N in the previous study; this would be consistent with our result that combined N, P and K addition increased the abundances of nirK, nirS and nosZ but had no detectable effect on nifH gene abundances (**Fig. 2 h**). Interestingly, differential responses of nitrification, denitrification and N fixation genes to N addition, the combination of N and P and the combination of N, P and K addition (**Fig. 2e-h**) were observed; these results suggest that nitrifier, denitrifier and N fixer community growth was also limited by these nutrients because N application significantly increased soil NH_4^+ and NO_3^-, which may cause a shift in nutrient limitation in the microbial community from N to other nutrients (Dong et al., 2015). It has also been reported that soil microbial communities lag climate change by approximately 50 years due to the comparatively slow impact of climate change factors on soil edaphic properties (Ladau et al., 2018). For this reason, GCFs that more rapidly induce changes in key edaphic properties are most likely to result in larger and more consistent effects on the soil microbiome.

Factors controlling the responses of soil archaeal, bacterial and N-cycling gene abundances to GCFs

The responses of soil archaeal, bacterial and N-cycling gene abundances to GCFs also depended on the habitat (biome) (**Figs. 3 and S5**), duration and magnitude of manipulation (**Table S5**), and the N form provided by N addition (**Table S3&S4**). These findings are consistent with previous studies (Carey et al., 2016; Xu and Yuan, 2017; Ouyang et al., 2018; Dai et al., 2020; Jia et al., 2020), as the spatial location,

climate, edaphic factors and habitat play important roles in microbial niche differentiation. eCO_2 significantly decreased the nifH gene abundances in upland ecosystems (most of the studies were conducted in grasslands) (**Figs. 3a and S5**), in agreement with the results of Yang et al. (2019) from grasslands, but contrasted with the results of a previous study reporting that eCO_2 increased the abundance of the nifH gene in grasslands after 12 years of CO_2 exposure (Tu et al., 2017). This may have been due to eCO_2 not causing NH_4^+ depletion (**Table S6**). In addition, the decreased nirK abundance under warming may have been caused mainly by the greater decrease in the nirK gene in agricultural soils; however, warming increased the nirK gene abundance in grasslands (**Fig. S5b**), which may be related to the differential effect on soil NO_3^- in grasslands (14.7%) and agricultural soils (-9.9%). These results indicate the inconsistent response of the nirK gene in grasslands and agricultural ecosystems due to their different N sources (soil organic matter, inorganic fertilizer) (Huhe et al., 2016). Interestingly, bacterial and N-cycling gene abundances responded positively to N addition in lowland soils, whereas mostly negative effects were observed in upland soils. These results suggest that soil microbial communities may exhibit different strategies in response to N addition in upland and lowland soils. One explanation for this phenomenon could be related to a larger increase in SOC in lowland (9.1%) than in upland (2.7%) soils under N addition because of the larger inputs of root exudates and plant residues and lower decomposition rates in lowland soils (Wei et al., 2021). In addition, although N addition relieves N limitation in both uplands and lowlands (soil NH_4^+ and NO_3^- increased by 18% and 71% in lowlands and by 71% and 90% in uplands, respectively), a larger decrease in soil pH in uplands (6%) than in lowlands (2%) may also lead to different responses of microbes and N-cycling genes to N addition (**Fig. 3**).

When the data from all GCF studies were pooled, we found that the RRs of soil archaeal, bacterial and N-cycling gene abundances were correlated with the RRs of soil pH and nutrient availability (**Table S7**), consistent with previous findings (Bru et al., 2011; Gubry-Rangin et al., 2011; Yao et al., 2013; Wei et al., 2020). In this study, significantly positive relationships between the RRs of soil microbes and N-cycling genes and soil pH were observed, except for a negative correlation between the RRs of amoA-AOB and pH (**Fig. 5**). Although the most important factors driving soil

bacterial, archaeal and N-cycling gene abundances differed worldwide in steady-state soils (control without GCF treatments) (**Fig. 1 c-j**), under GCF manipulations, the change in soil pH was the most important factor driving the change in soil microbes and N-cycling genes, except the nifH gene (**Fig. 4**). These findings are consistent with previous studies at local and regional scales (Bru et al., 2011; Hu et al., 2013; Stempfhuber et al., 2015) and supports our understanding of the role that soil pH plays in influencing soil microbial growth (Bartram et al., 2014; Zhalnina et al., 2015) and N-cycling microbial communities (Yang et al., 2014; Ouyang et al., 2018). This may have occurred because some of the GCF manipulation treatments resulted in a rapid alteration of edaphic properties disrupting the steady-state condition and so that microbial community re-assembly occurs rather than microbial adaptation. In this context, soil pH change became the most important factor influencing the change in functional gene abundance. However, a decline in effect size of soil pH did not entirely imply a decrease in effect size of archaeal, bacterial, and N-cycling gene abundances under GCF manipulation treatments; for example, decreases in only archaeal, nosZ and nifH gene abundances were observed under N addition (**Fig. 2 e**), and decreases in only amoA-AOA and nifH gene abundances were observed under combined N and P addition (**Fig. 2 g**) with significantly decreased soil pH. In contrast, combined N, P and K addition decreased the soil pH but increased the abundances of most of the N-cycling genes (**Fig. 2 h**). These results suggest that the responses of microbes and N-cycling genes to nutrient addition may depend on the balance between the promoting effect of the increase in soil substrate availability (SOC, NH_4^+ and NO_3^-) and the possible inhibitory effect of the decrease in pH under nutrient addition. This interaction requires further investigation.

Linkages between N-cycling gene abundances and N transformation processes

In this study, various responses of N-cycling process rates to GCFs were observed (**Fig. 6 and Table S6**). When compared across all datasets under GCFs, the RR of AOA, AOB, nirS and nosZ were significantly correlated with the corresponding process rates, which is consistent with previous studies (Rocca et al., 2015; Carey et al., 2016; Ouyang et al., 2018) and indicates that nitrification and denitrification have a stronger correlation with gene abundance than other processes. In addition, we found that a significant increase in the PNR was observed when the abundance of the

amoA-AOB gene increased under N, N×P, and N×P×P addition but that the PNR did not change when GCFs increased or decreased the abundance of amoA-AOA rather than that of amoA-AOB (**Figs. 2 and 6**). These results indicate that although positive correlations between the RRs of amoA-AOA and amoA-AOB and PNR were observed, a change in amoA-AOB might contribute more to an increase in PNR than a change in amoA-AOA under GCFs mainly as a result of the nutrient addition manipulations (**Fig. 7**). This result is consistent with previous studies (Di et al., 2009; Jia and Conrad, 2009; Tao et al., 2017) reporting that amoA-AOB was functionally vital for nitrification in nutrient-rich agricultural soil and consistent with the sometimes mixotrophic lifestyle of AOA. Moreover, we also found that N transformation processes were correlated with the duration and magnitude of manipulation and with changes in soil properties (**Tables S8 and S9**). Although N cycling gene abundance is assumed to be directly linked with N transformation processes, changes of overall patterns in the N-cycling gene abundance under GCFs were in some cases inconsistent with N transformation process rates (**Figs. 2 and 6, Table S6**). For example, PPT+ significantly increased denitrifying gene abundance (**Fig. 2c**) but had no effects on DEA and N_2O emissions (**Fig. 6c**), which might be due to delayed or limited expression of relevant genes, because lots of DNA genes in soil microbial community were dormant on from microbial residues (Dai et al., 2020). Moreover, although a positive relationship between RR of DEA and nirS genes was observed (**Fig. 7c**), we did not find synchronous changes of effect size between denitrifier abundance and DEA or N_2O emissions (**Fig. 7h-j**), indicating that different denitrifiers are likely responsible for the denitrification process under GCFs. Thus, our dataset provides evidence that the nitrification process remains relatively stable under manipulated climate changes but is significantly simulated by nutrient addition. These results suggest an increase challenge of understanding the responses of the nitrification and denitrification process under ongoing global change.

Conclusions

The meta-analysis presented here explored changes in the abundances of soil bacteria, archaea and key N-cycling genes along with N transformation processes and soil physicochemical properties under GCFs. Overall, the abundances of all N-cycling

genes except nirK were relatively resistant to eCO_2, warming and water reduction, and may be related to the time required to to alter edaphic properties under these GCFs, whereas water addition strongly activated multiple N-cycling genes in parallel with stronger impacts on soil edaphic properties like pH. Moreover, N addition and the combination of N addition with other treatments significantly increased the abundance of amoA-AOB genes. These results suggest that global change has a profound effect on ecosystems experiencing increases in precipitation and N deposition. Although soil microbes and N-cycling genes were controlled by different factors across climates, spatial locations, and habitats (upland and lowland) under natural conditions, GCFs influences soil microbial abundance and N-cycling genes predominantly associated with through changes in soil pH. The habitat (upland or lowland) and the duration and magnitude of the manipulation also contributed to the variabilities in soil microbe and N-cycling genes under different GCFs. In addition, the RRs of AOA, AOB, nirS and nosZ were significantly correlated with their corresponding process rates, and GCF-induced shifts in PNR were well explained by changes in amoA-AOB. However, the abundances of changes in denitrification genes were repressed or activated under some GCF treatments but were not reflected in the corresponding N transformation process rates. Together, these results demonstrate that GCF impacts on soil pH should be carefully considered in causal analyses and modeling of the impacts on soil microbiomes and N cycling processes. Further, we show that interactions with elevation or topographic position, as well as biome type and land use alters how GCF manipulations impact soil microbiomes. This highlights the need for a microbe-explicit mechanistic representation of these interactions in models that aim to predict the future trajectory of soil biogeochemical cycles under ongoing global change.

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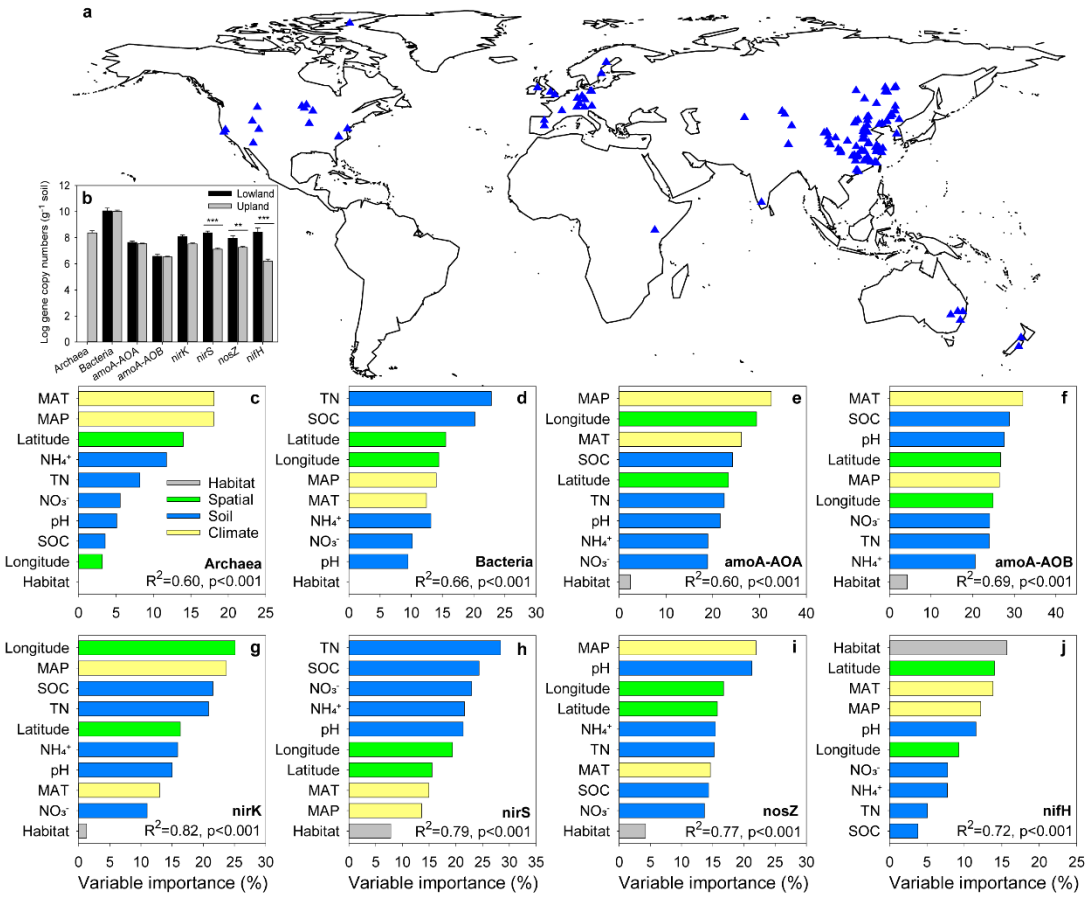

Figure 1 Distribution of abundance of soil microbe and key N-cycling genes. (a) Global distributions of experiments reporting abundance of archaea, bacteria, and key N-cycling genes under global change factors in this meta‐analysis. (b) Abundance of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH) in upland and lowland habitat; ** and *** indicate significant difference between upland and lowland at $p<0.01$ and $p<0.001$, respectively. (c) Variable importance of moderators for abundance of archaea, bacteria, and key N-cycling genes under natural conditions without global change treatment. The importance values are derived from a weighted random-forest analysis including variables as moderators in the model, and R^2 is the variability of the abundance of archaea, bacteria, and key N-cycling genes explained by the weighted random-forest model; MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen.

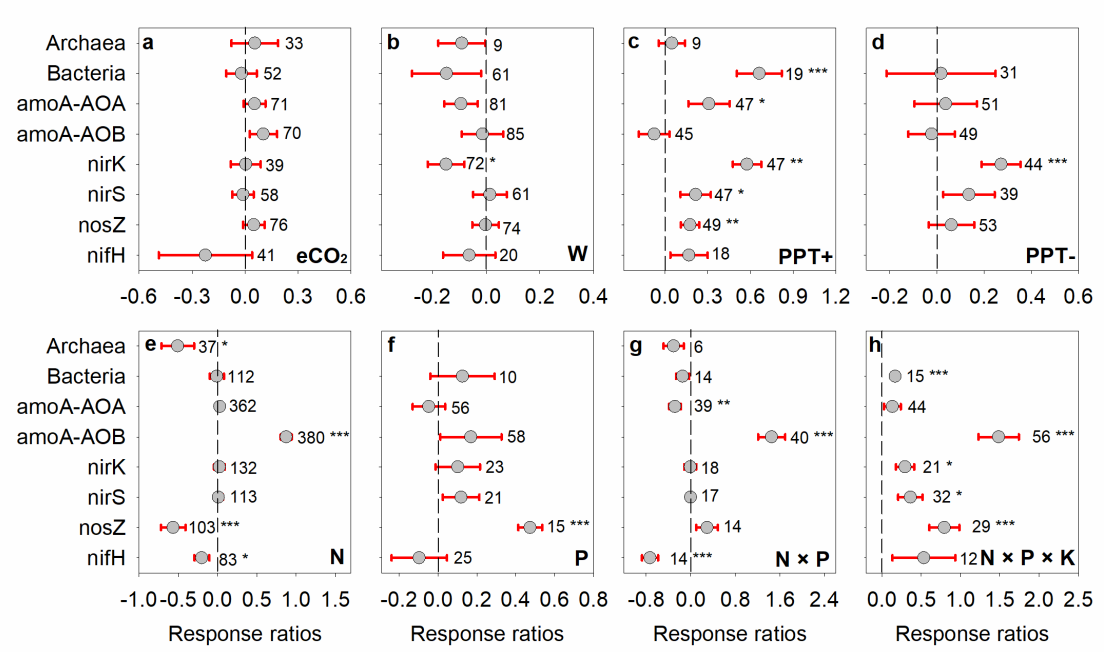

Figure 2 The weighted effect size of global change factors (enrichment of CO_2 (eCO_2), warming, water addition (PPT+), water reduction (PPT-), nirtrogen (N) addition, phosphorus (P) addition, N plus P addition (N×P), and c N plus P plus potassium (K) addition (N×P×K)) on abundance of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH. The values next to the bars are the corresponding numbers of observations. Error bars represent 95% confidence intervals (CIs). *, ** and *** indicate significant effect size to warming, as the 95%, 99% and 99.9% CIs of the effect size did not overlap zero, respectively.

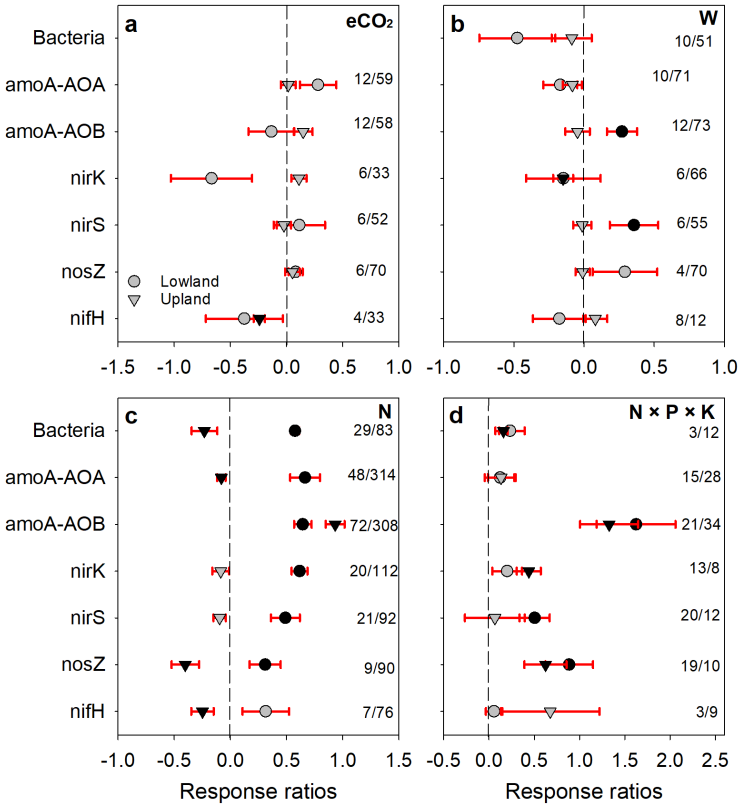

Figure 3 The weighted effect size of eCO_2 (a), warming(b), N addition(c), and N×P×K addition (d) on abundance of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH in upland and lowland habitat, respectively. The values next to the bars are the corresponding numbers of observations in lowland and upland, respectively; the solid black dots indicate significant effect size compare with zero.

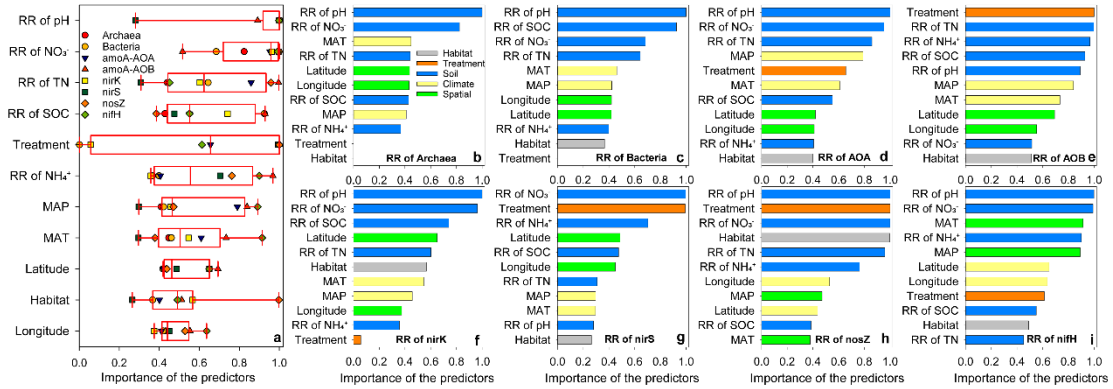

Figure 4 Relative importance of the predictors to response of archaea, bacteria, and key N-cycling genes to global change factors. (a) Model-averaged importance of the predictors for response ratios (RR) of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH). Boxplots show whiskers (denote the lowest and highest values), quartiles, and median of model-averaged importance for different predictors. (b-i) Relative importance of the predictors of global changes effect on response ratios of archaea, bacteria, and key N-cycling genes. The importance is based on the sum of Akaike weights derived from the model selection using AIC. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen.

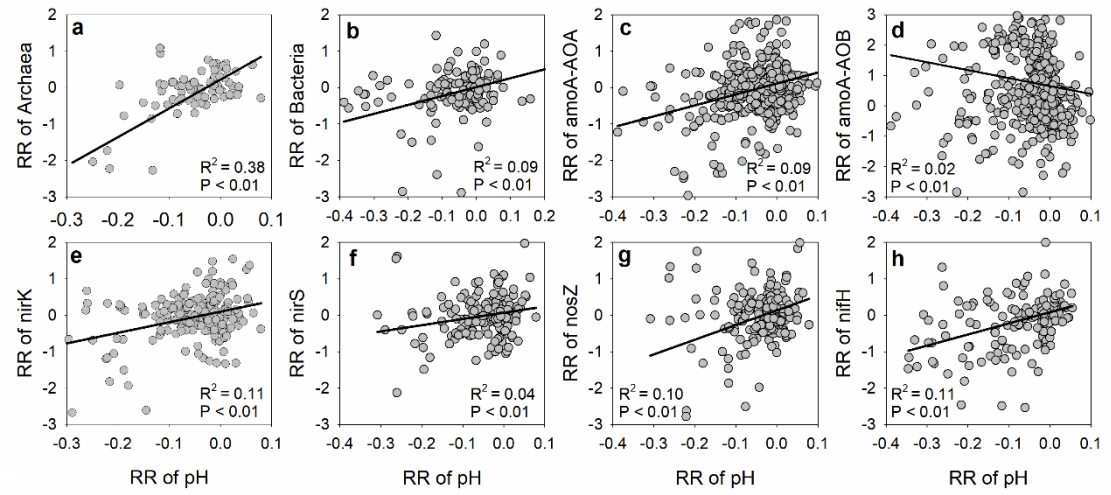

Figure 5 Relationships between response ratio of soil pH and archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH).

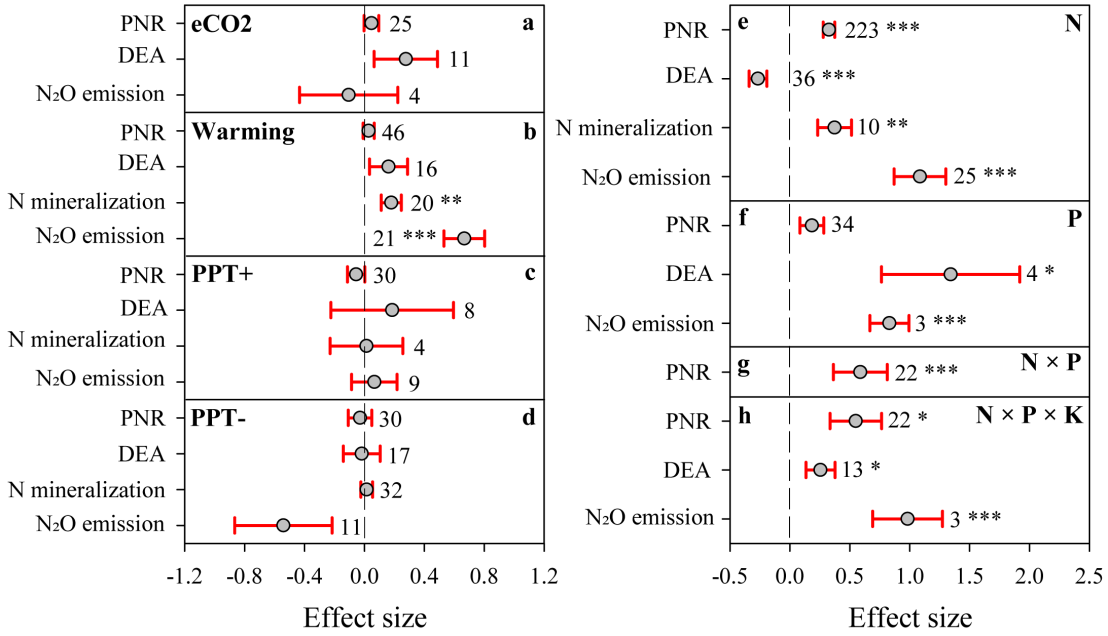

Figure 6 The weighted effect size of global change factors on nitrogen transformation processes. The values next to the bars are the corresponding numbers of observations. Error bars represent 95% confidence intervals (CIs). *, ** and *** indicate significant effect size to warming, as the 95%, 99% and 99.9% CIs of the effect size did not overlap zero, respectively. PNR, potential nitrification rate; DEA: denitrification enzyme activity.

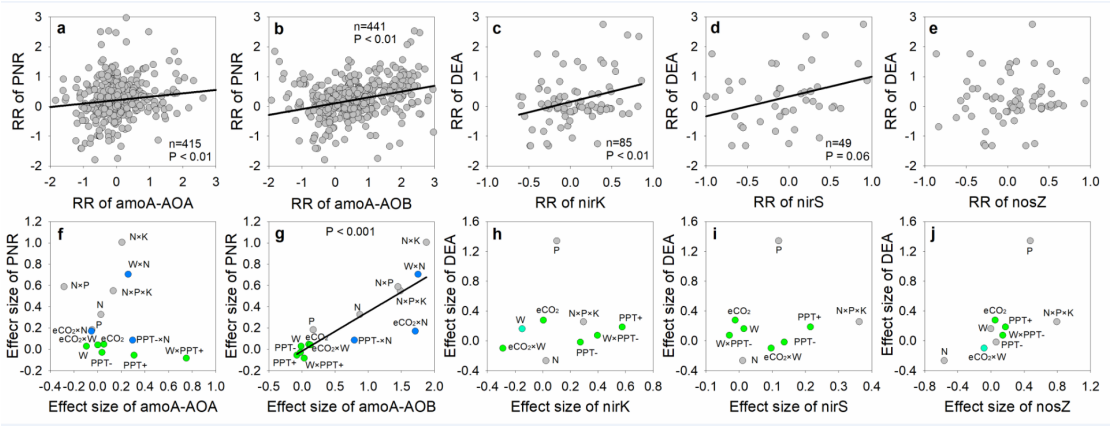

Figure 7 Relationships between response ratios of nitrogen transformation processes and key N-cycling genes. PNR, potential nitrification rate; DEA, denitrification enzyme activity; eCO_2, enrichment of CO_2; W, warming; PPT+, water addition; PPT-, water reduction; N, nitrogen addition; P, phosphorus addition; N×P, N plus P addition; N×P×K, N plus P plus potassium (K) addition; eCO_2×N: eCO_2 plus N; eCO_2×W: eCO_2 plus warming; W×PPT+, warming plus PPT+; PPT-×N: PPT- plus N addition; W×N, warming plus N addition; N×K, N plus K addition.

Supplementary Materials

Figure S1 Kernel density estimates (smoothed version of the histogram) for response ratio of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH).

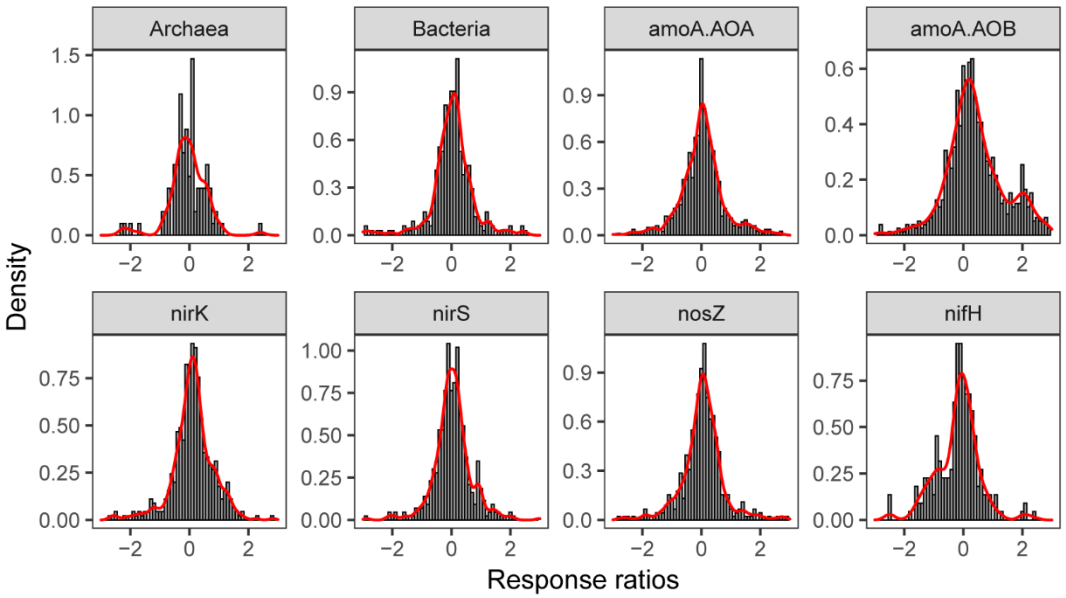

Figure S2 Abundance of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH) in different biomes; lowercase letters indicate significant difference among biomes at $p<0.05$.

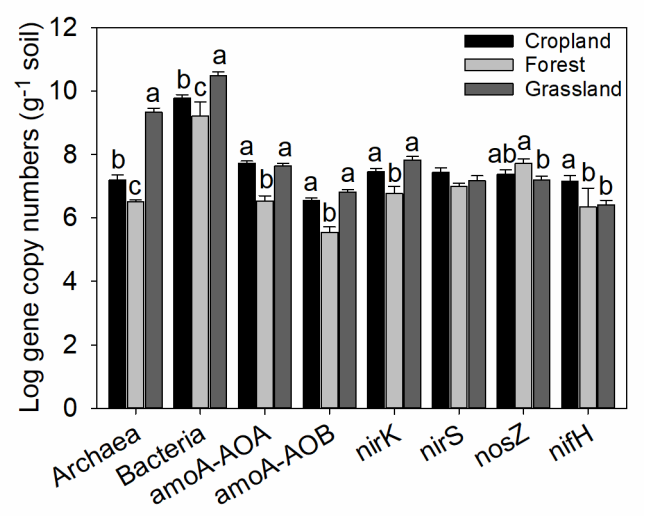

Figure S3 The weighted effect size of global change factors on abundance of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH). (a) combination of warming and nitrogen (N) addition (warming×N); (b) combination of warming and water reduction (warming×PPT-); (c) combination of warming and water addition (warming×PPT+); (d) combination of enrichment of CO_2 (eCO_2) and warming (eCO_2×warming); (e) combination of N and potassium (K) addition (N×K), N and PPT- (N×PPT-, and N and eCO_2 (N×eCO_2); (f) combination of N and PPT+ (N×PPT+). The values next to the bars are the corresponding numbers of observations. Error bars represent 95% confidence intervals (CIs). *, ** and *** indicate significant effect size to warming, as the 95%, 99% and 99.9% CIs of the effect size did not overlap zero, respectively.

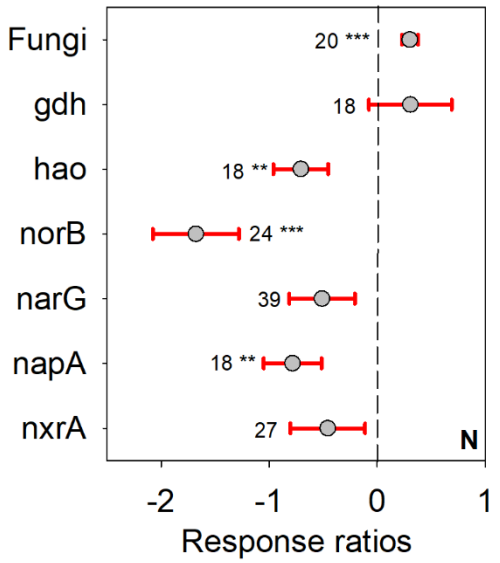

Figure S4 The weighted effect size of N addition on abundance of fungi and N-cycling genes.

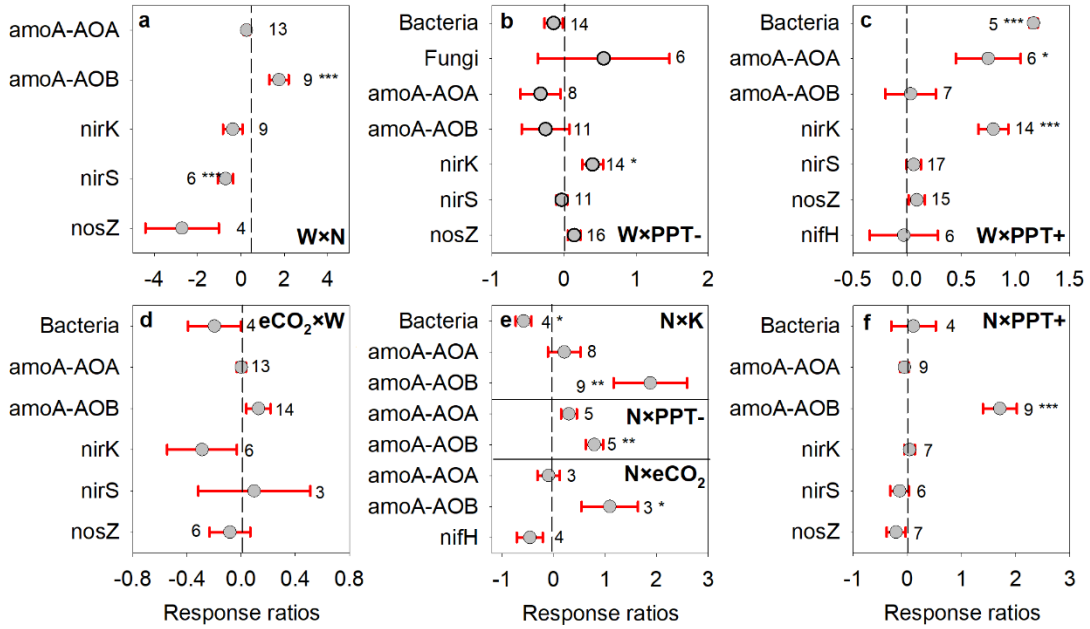

Figure S5 The weighted effect size of global change factors on abundance of archaea, bacteria, and key N-cycling genes in different biomes. The values next to the bars are the corresponding numbers of observations in cropland, forest, and grassland, respectively; the solid black dots indicate significant effect size compare with zero.

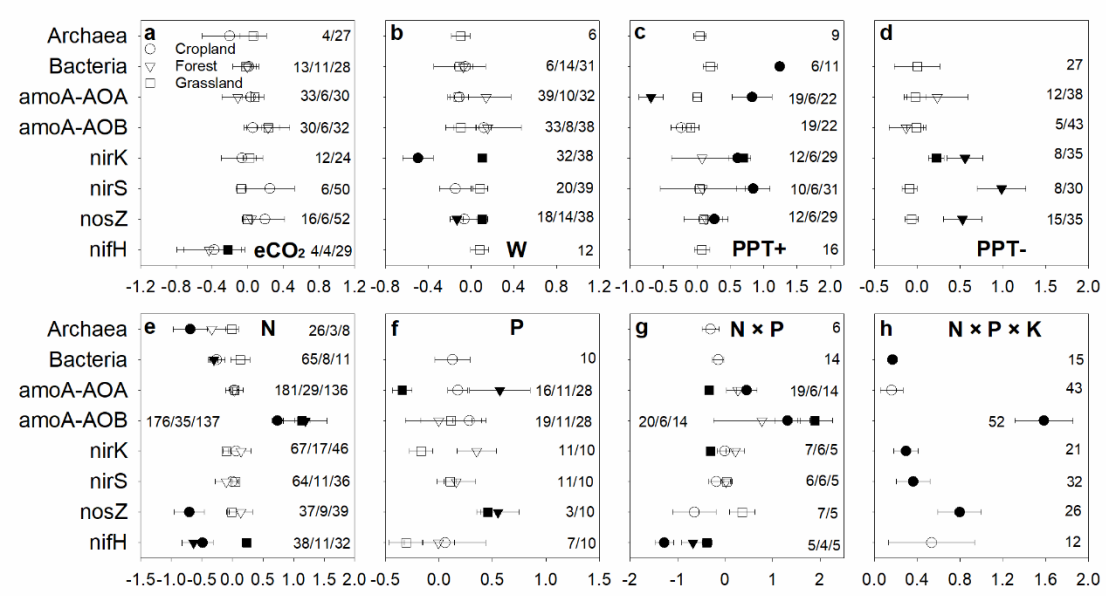

Figure S6 Relative importance of the predictors of global changes effect on response ration of archaea, bacteria, and key N-cycling genes. The importance is based on the sum of Akaike weights derived from the model selection using AIC. A cutoff of 0.8 is set to differentiate between important and non-essential predictors. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen.

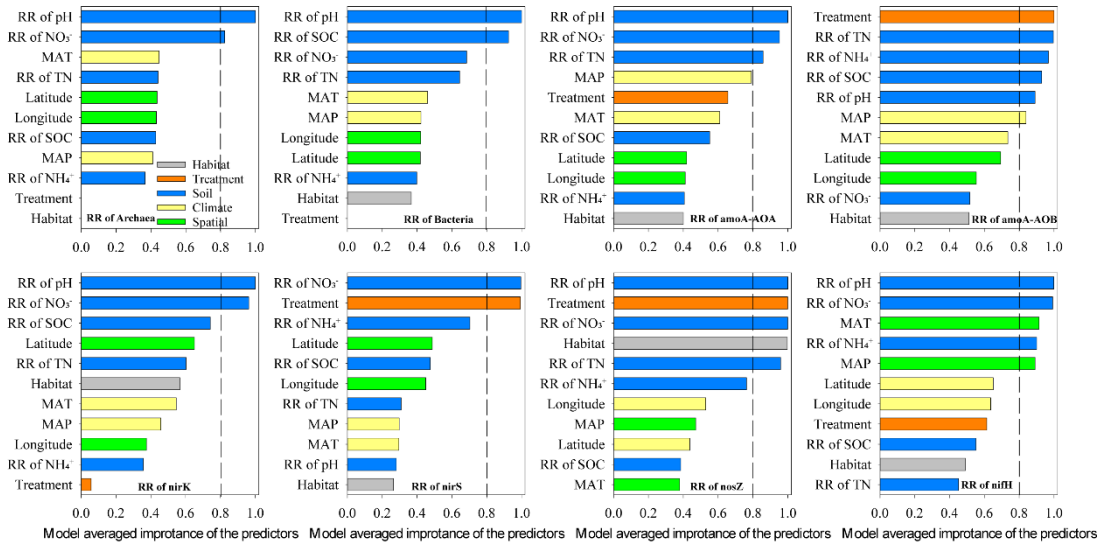

Figure S7 Relationships between response ratio of nirK and changes in precipitation.

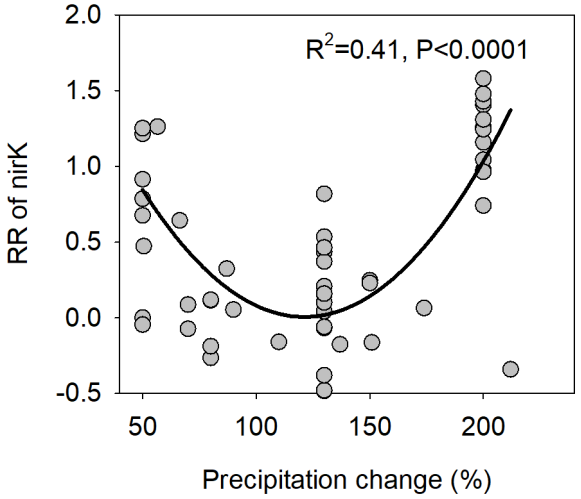

Table S1 Main target variables involved in this study.

Name of variable	Unit
Coordinates (latitude and longitude)	°
Mean annual temperature	°C
Mean annual precipitation	mm
Warming intensity	°C
eCO_2	ppm
Precipitation change	%
N rate	kg ha^{-1}
P rate	kg ha^{-1}
Experimental duration	Month
Soil temperature	°C
Soil moisture	%
Soil organic C (SOC)	g kg^{-1}
Soil dissolved organic C (DOC)	mg kg^{-1}
Soil microbial biomass C (MBC)	mg kg^{-1}
Soil total N (TN)	g kg^{-1}
Soil NH_4^+-N	mg kg^{-1}
Soil NO_3^--N	mg kg^{-1}
Soil microbial biomass N (MBN)	mg kg^{-1}
Soil available N	mg kg^{-1}
Soil dissolved organic N (DON)	mg kg^{-1}
Archaea	copy g^{-1} soil
Bacteria	copy g^{-1} soil
Fungi	copy g^{-1} soil
amoA-AOA gene	copy g^{-1} soil
nirS gene	copy g^{-1} soil
nirK	copy g^{-1} soil
nosZ	copy g^{-1} soil
nifH	copy g^{-1} soil
Potential nitrification rate (PNR)	mg NO_2-N h^{-1} kg^{-1} soil
Denitrification enzyme activity (DEA)	μg N_2O h^{-1} g^{-1} soil
N_2O flux	μg m^{-2} h^{-1}

Table S2 Pearson's r correlation coefficients among among environment factors and

archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH) under natural condition without global change treatment.

Variable	Archaea	Bacteria	AOA	AOB	nirK	nirS	nosZ	nifH
Latitude	**0.36****	-0.04	-0.02	-0.01	**0.51****	**-0.13***	0.04	**-0.29****
Longitude	-0.12	**-0.14***	**0.27****	-0.05	**-0.41****	**0.42****	**0.29****	**0.38****
MAT	**-0.38****	-0.10	**-0.15*****	-0.04	**-0.14***	**-0.20****	**-0.22****	-0.05
MAP	**-0.24***	**-0.17****	**-0.24*****	**-0.22*****	**-0.27****	**0.20****	**0.18****	**0.27****
SOC	**-0.38***	**-0.26****	**-0.17*****	**-0.14***	**0.20***	-0.01	**-0.20***	0.01
TN	0.00	**-0.38****	-0.12	-0.03	**0.24***	-0.11	**-0.24***	0.07
NH_4^+	0.05	0.02	-0.03	**-0.13***	0.02	**0.22****	-0.11	**0.25***
NO_3^-	-0.22	0.13	0.09	**0.20****	**0.27****	0.08	0.13	-0.11
pH	**0.44****	0.11	**0.33****	**0.25****	**0.31****	**0.33****	**0.43****	**0.37****
Archaea	1.00	**0.50****	**0.75****	**0.72****	**0.73****	**0.63****	**0.77****	**0.89****
Bacteria		1.00	**0.49****	**0.52****	**0.28****	**0.56****	**0.71****	0.15
AOA			1.00	**0.35****	**0.17***	**0.64****	**0.50****	**0.33***
AOB				1.00	**0.42****	**0.53****	**0.69****	**0.52****
nirK					1.00	**0.35****	**0.43****	**0.38****
nirS						1.00	**0.85****	**0.88****
nosZ							1.00	**0.96****

* and ** indicate a significant relationship at $p<0.05$ and $p<0.01$, respectively. MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen.

Table S3 The between-group heterogeneity (Qb) of mean annual temperature (MAT) mean annual precipitation (MAP), habitat, nitrogen (N) form and rate and experimental duration on response of archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH).

Response variables	Variables	df_b	Qb	*P*-Value
Bacteria	MAT	1	0.299	0.585
	MAP	1	2.887	0.089
	Duration	1	1.325	0.250
	N rate	1	0.003	0.956
	Habitat	1	0.374	0.541
	N form	1	0.012	0.911
amoA-AOA	MAT	1	0.028	0.867
	MAP	1	0.051	0.821
	Duration	1	45.508	**0.000**
	N rate	1	2.058	0.151
	Habitat	1	0.486	0.486
	N form	3	8.597	**0.035**
amoA-AOB	MAT	1	32.230	**0.000**
	MAP	1	9.860	**0.002**
	Duration	1	6.660	**0.010**
	N rate	1	12.760	**0.000**
	Habitat	1	7.290	**0.007**
	N form	3	3.920	0.270
nirK	MAT	1	3.616	0.057
	MAP	1	1.555	0.212
	Duration	1	9.309	**0.002**
	N rate	1	2.225	0.136
	Habitat	1	2.776	0.096
	N form	2	0.424	0.809
nirS	MAT	1	1.853	0.173
	MAP	1	0.799	0.371
	Duration	1	1.294	0.255
	N rate	1	0.205	0.650
	Habitat	1	7.041	**0.008**
	N form	2	3.505	0.173
nosZ	MAT	1	0.006	0.937
	MAP	1	3.021	0.082
	Duration	1	16.584	**0.000**
	N rate	1	0.390	0.533
	Habitat	1	2.771	0.096
	N form	2	1.456	0.483

Habitat and N form are categorical variables. *P*-values in bold are statistically significant at P < 0.05.

Table S4 Weighted effect size of archaea, bacteria, and key N-cycling genes, edaphic factors, and N transformation processes under different N forms.

N form	Variable	df	Effect sizes	SE	Pooled variance
Urea	Archaea	24	-0.76	0.29	**<0.01**
	Bacteria	89	0.02	0.11	>0.05
	amoA-AOA	173	-0.12	0.07	>0.05
	amoA-AOB	200	0.75	0.09	**<0.01**
	nirK	54	-0.11	0.14	>0.05

	nirS	52	-0.19	0.09	**<0.05**
	nosZ	32	-0.83	0.27	**<0.01**
	nifH	40	-0.50	0.17	**<0.01**
	pH	153	-0.07	0.01	**<0.01**
	SOC	127	0.04	0.01	**<0.01**
	TN	90	0.04	0.01	**<0.01**
	NH_4^+	145	0.75	0.10	**<0.01**
	NO_3^-	156	1.13	0.09	**<0.01**
	PNR	131	0.36	0.07	**<0.01**
	N_2O	10	0.53	0.27	>0.05
NH_4NO_3	Archaea	11	-0.03	0.10	>0.05
	Bacteria	16	0.05	0.12	>0.05
	amoA-AOA	125	0.07	0.03	**<0.05**
	amoA-AOB	113	1.06	0.15	**<0.01**
	nirK	60	-0.02	0.06	>0.05
	nirS	44	-0.01	0.06	>0.05
	nosZ	61	-0.09	0.09	>0.05
	nifH	38	0.03	0.10	>0.05
	pH	97	-0.05	0.01	**<0.01**
	SOC	89	0.02	0.01	>0.05
	TN	69	0.05	0.01	**<0.01**
	NH_4^+	113	0.26	0.07	**<0.01**
	NO_3^-	123	0.68	0.08	**<0.01**
	PNR	70	0.21	0.05	**<0.01**
	DEA	35	-0.30	0.07	**<0.01**
	N_2O	9	1.18	0.42	**<0.01**
NH_4^+	amoA-AOA	28	0.08	0.12	>0.05

	amoA-AOB	32	1.10	0.20	**<0.01**
	nirK	3	0.42	0.27	>0.05
	nirS	4	0.14	0.43	>0.05
	nosZ	4	0.33	0.19	>0.05
	pH	18	-0.01	0.00	**<0.01**
	SOC	17	0.05	0.02	**<0.05**
	TN	13	0.07	0.03	**<0.05**
	NH_4^+	18	0.15	0.08	**<0.05**
	NO_3^-	26	0.70	0.13	**<0.01**
	PNR	12	0.58	0.23	**<0.05**
	N_2O	4	1.18	0.41	**<0.01**
	amoA-AOA	5	0.23	0.13	>0.05
	amoA-AOB	6	0.16	0.30	>0.05
	pH	7	0.00	0.01	>0.05
NO_3^-	SOC	5	0.08	0.04	>0.05
	NH_4^+	4	0.02	0.09	>0.05
	NO_3^-	4	0.89	0.46	>0.05
	PNR	4	0.22	0.27	>0.05

df, corresponding numbers of paired observations; pooled variances <0.05 indicate significant difference compare with zero.

Table S5 Pearson's r correlation coefficients among GCFs induced shifts in archaea, bacteria, and key N-cycling genes and magnitude and duration of GCF.

Treatment	Variables	Archaea	Bacteria	amoA-AOA	amoA-AOB	nirK	nirS	nosZ	nifH
eCO_2	Duration	-0.21	-0.02	0.09	0.13	**-0.40***	-0.07	0.00	0.19
	Increased CO_2	0.17	-0.03	0.04	-0.15	**-0.37***	-0.03	0.04	-0.22
Warming	Duration	-0.06	-0.01	-0.12	-0.16	-0.30	-0.19	-0.16	
	Increased temperature		0.02	0.16	-0.20	0.13	0.11	-0.16	**0.62****
PPT+	Duration		0.17	0.31	-0.22	0.46	0.16	0.19	0.15
	Increased precipitation	-0.18	0.15	0.26	0.06	**0.74****	-0.10	0.09	-0.18
PPT-	Duration		-0.06	0.00	-0.27	-0.56	-0.10	-0.41	
	Decreased precipitation		-0.03	-0.15	0.12	**-0.53***	0.19	-0.13	
N	Duration	-0.16	**-0.26***	**-0.25****	**-0.13***	**-0.29****	**-0.25***	**-0.33****	**-0.47****
	N rate	-0.11	0.00	**-0.22****	-0.01	0.00	-0.17	**-0.21***	0.03
P	Duration		-0.43	**0.29***	0.03	0.18	0.00	**0.55***	0.22
	P rate			0.09	-0.24	0.14	-0.06	0.09	0.18
N×P×K	Duration		0.27	-0.10	**0.58****	-0.10	-0.09	0.17	**0.86****
N×P	Duration		-0.06	0.05	0.07	**-0.64***	-0.58	**-0.90****	**-0.67***

*, $P<0.05$; **, $P<0.01$.

Table S6 Weighted effect size of edaphic factors, and N transformation processes under global change factors.

Treatment	Variable	df	Response ratios	SE	Pooled variance
eCO_2	ST	6	0.00	0.01	>0.05
	SM	16	0.00	0.02	>0.05
	pH	35	0.00	0.00	>0.05
	SOC	25	0.05	0.02	**<0.05**
	DOC	29	-0.04	0.05	>0.05
	TN	29	-0.01	0.02	>0.05
	NH_4^+	68	0.02	0.05	>0.05
	NO_3^-	64	-0.07	0.04	>0.05
	DON	22	-0.08	0.03	**<0.01**
	MBC	8	0.22	0.18	>0.05
	MBN	4	0.07	0.18	>0.05
Warming	ST	30	0.12	0.02	**<0.01**
	SM	46	-0.06	0.02	**<0.01**
	pH	46	-0.01	0.00	>0.05
	SOC	36	0.00	0.01	>0.05
	DOC	8	0.13	0.03	**<0.01**
	TN	34	-0.01	0.01	>0.05
	NH_4^+	72	-0.10	0.03	**<0.01**
	NO_3^-	90	0.03	0.06	>0.05
	AN	12	0.40	0.07	**<0.01**
	DON	18	0.11	0.07	>0.05
	MBC	10	0.03	0.15	>0.05
	MBN	16	0.00	0.08	>0.05
	NEA	4	0.28	0.10	**<0.01**
PPT+	SM	40	0.20	0.03	**<0.01**
	pH	30	0.03	0.00	**<0.01**
	SOC	22	0.01	0.01	>0.05
	TN	28	0.00	0.02	>0.05
	NH_4^+	40	0.12	0.08	>0.05
	NO_3^-	40	-0.15	0.09	>0.05
	MBC	6	0.13	0.13	>0.05
PPT-	ST	6	0.02	0.01	>0.05
	SM	32	-0.31	0.07	**<0.01**
	SOC	6	-0.17	0.13	>0.05
	DOC	5	-0.12	0.09	>0.05
	TN	8	-0.09	0.10	>0.05
	NH_4^+	38	-0.07	0.10	>0.05
	NO_3^-	51	-0.15	0.13	>0.05
	AN	12	0.05	0.05	>0.05
	DON	13	0.06	0.04	>0.05
	MBC	6	-0.02	0.10	>0.05
	MBN	9	-0.11	0.10	>0.05
	NEA	4	-0.03	0.10	>0.05
N	ST	10	-0.04	0.01	**<0.01**

	SM	88	0.00	0.00	>0.05
	pH	278	-0.06	0.00	**<0.01**
	SOC	241	0.03	0.01	**<0.01**
	DOC	15	0.08	0.05	>0.05
	TN	174	0.04	0.01	**<0.01**
	NH_4^+	286	0.47	0.06	**<0.01**
	NO_3^-	317	0.96	0.06	**<0.01**
	AN	39	0.14	0.01	**<0.01**
	DON	14	0.37	0.09	**<0.01**
	MBC	67	0.28	0.04	**<0.01**
	MBN	64	0.34	0.04	**<0.01**
	NEA	3	0.15	0.12	>0.05
P	SM	21	0.03	0.01	**<0.05**
	pH	48	0.00	0.01	>0.05
	SOC	45	0.04	0.02	**<0.05**
	DOC	8	0.17	0.10	>0.05
	TN	42	0.02	0.02	>0.05
	NH_4^+	43	-0.10	0.06	>0.05
	NO_3^-	45	-0.06	0.05	>0.05
	AN	9	1.52	0.32	**<0.01**
	DON	4	0.21	0.13	>0.05
N×P×K	pH	45	-0.07	0.01	**<0.01**
	SOC	46	0.11	0.02	**<0.01**
	DOC	11	0.21	0.13	>0.05
	TN	36	0.11	0.02	**<0.01**
	NH_4^+	36	0.33	0.15	**<0.05**
	NO_3^-	41	0.55	0.13	**<0.01**
	AN	3	0.33	0.07	**<0.01**
	DON	11	-0.03	0.05	>0.05
	MBC	8	-0.06	0.09	>0.05
	MBN	10	-0.03	0.12	>0.05
N×P	SM	11	0.05	0.03	>0.05
	pH	35	-0.09	0.02	**<0.01**
	SOC	33	0.11	0.02	**<0.01**
	DOC	4	0.18	0.10	>0.05
	TN	31	0.10	0.02	**<0.01**
	NH_4^+	32	0.44	0.19	**<0.05**
	NO_3^-	33	0.93	0.11	**<0.01**
	AN	5	1.65	0.42	**<0.01**
W×N	SM	8	-0.03	0.06	>0.05
	pH	4	-0.09	0.04	**<0.05**
	SOC	4	0.06	0.04	>0.05
	TN	4	0.09	0.05	>0.05
	NH_4^+	8	0.73	0.31	**<0.05**
	NO_3^-	9	1.99	0.36	**<0.01**
	PNR	8	0.71	0.13	**<0.01**
	N min	3	0.88	0.20	**<0.01**
	N_2O	3	1.25	0.27	**<0.01**

W×PPT-	ST	5	0.27	0.04	**<0.01**
	SM	5	-0.18	0.09	>0.05
	NH_4^+	13	0.42	0.17	**<0.01**
	NO_3^-	6	0.49	0.11	**<0.01**
	DON	6	0.25	0.10	**<0.05**
	MBN	3	-0.32	0.26	>0.05
	NEA	4	0.26	0.13	**<0.05**
	DEA	5	0.07	0.04	**<0.05**
	N min	6	0.16	0.13	>0.05
W×PPT+	SM	5	0.07	0.08	>0.05
	pH	3	0.02	0.01	>0.05
	SOC	4	-0.01	0.04	>0.05
	TN	3	-0.02	0.04	>0.05
	NH_4^+	7	0.17	0.11	>0.05
	NO_3^-	7	-0.29	0.24	>0.05
	PNR	5	-0.08	0.11	>0.05
eCO_2×W	SM	8	-0.02	0.01	>0.05
	pH	14	0.00	0.00	>0.05
	SOC	8	0.01	0.01	**<0.01**
	DOC	3	0.30	0.11	**<0.01**
	TN	9	-0.04	0.02	>0.05
	NO_3^-	17	-0.10	0.08	>0.05
	PNR	12	0.04	0.01	**<0.01**
	DEA	4	-0.10	0.13	>0.05
N×PPT+	SM	6	0.24	0.10	**<0.05**
	pH	6	-0.05	0.01	**<0.01**
	SOC	5	0.02	0.05	>0.05
	TN	5	0.00	0.03	>0.05
	NH_4^+	6	0.01	0.08	>0.05
	NO_3^-	6	0.57	0.13	**<0.01**
	PNR	7	0.17	0.18	>0.05
N×PPT-	NH_4^+	3	0.83	0.93	>0.05
	NO_3^-	3	0.82	1.03	>0.05
	PNR	4	0.09	0.10	>0.05
	DEA	4	-0.23	0.11	**<0.05**
N×K	pH	6	-0.13	0.06	**<0.05**
	SOC	6	0.06	0.02	**<0.01**
	TN	4	0.12	0.03	**<0.01**
	NH_4^+	3	0.46	0.43	>0.05
	NO_3^-	4	1.05	0.44	**<0.05**
	PNR	6	1.01	0.55	>0.05

ST, soil temperature; SM, soil moisture; SOC, soil organic carbon; TN, total nitrogen, DOC, dissolved organic carbon; AN: available nitrogen; DON, dissolved organic N; MBC, microbial biomass C; MBN, microbial biomass N; PNR, potential nitrification rate; DEA: denitrification enzyme activity; NEA, nitrification enzyme activity; N min, N mineralization; N_2O, N_2O emission. df, corresponding numbers of paired observations; pooled variances <0.05 indicate significant difference compare with zero.

Table S7 Pearson's r correlation coefficients among geographical locations (latitude and longitude), climates (MAT and MAP), response ratio of soil physical-chemical properties (SOC, pH, NH_4^+, NO_3^- and TN) and response ratio archaea, bacteria, and key N-cycling genes (amoA-AOA, amoA-AOB, nirK, nirS, nosZ, and nifH) under global change factors.

Variable	Archaea	Bacteria	AOA	AOB	nirK	nirS	nosZ	nifH
Latitude	0.11	0.02	-0.02	**0.08***	**-0.14****	**-0.10***	0.04	-0.06
Longitude	-0.15	0.02	0.03	**0.12****	**-0.10***	0.04	-0.08	0.04
MAT	-0.11	0.05	**0.10****	-0.05	-0.06	0.09	0.05	-0.02
MAP	-0.16	-0.04	**0.10****	0.02	-0.03	0.03	**0.14****	**-0.17***
pH	**0.61****	**0.30****	**0.29****	**-0.13****	**0.33****	**0.21****	**0.31****	**0.33****
SOC	0.14	0.15	**0.16****	**0.22****	**0.28****	0.10	**0.23****	0.06
TN	0.09	**0.18***	**0.18****	**0.32****	**0.26****	0.12	**0.16***	-0.03
NH_4^+	**-0.26***	0.06	**-0.25****	**0.29****	0.05	-0.13	**-0.16****	**-0.28****
NO_3^-	0.03	-0.04	-0.07	**0.38****	0.10	0.05	-0.10	-0.09
Archaea	1.00	**0.66****	**0.67****	0.03	**0.65****	0.20	**0.49****	0.12
Bacteria		1.00	**0.39****	0.07	**0.73****	**0.30****	**0.59****	0.11
AOA			1.00	**0.19****	**0.46****	**0.52****	**0.36****	**0.47****
AOB				1.00	0.06	**0.13***	**0.13***	0.18
nirK					1.00	**0.40****	**0.31****	0.20
nirS						1.00	**0.47****	**0.40****
nosZ							1.00	**0.26****

MAT, mean annual temperature; MAP, mean annual precipitation; SOC, soil organic carbon; TN, total nitrogen. *, $P<0.05$; **, $P<0.01$.

Table S8 Pearson's r correlation coefficients among GCFs induced shifts in edaphic factors, N transformation processes, and magnitude and duration of GCF.

Treatment	Variables	pH	SOC	TN	NH_4^+	NO_3^-	PNR	DEA	N_2O
eCO_2	Duration	-0.42	0.17	0.23	**-0.35***	0.13	0.45	**0.68***	
	Increased CO_2	0.01	0.06	-0.21	0.08	-0.10	0.02	0.58	
Warming	Duration	-0.18	-0.06	0.10	-0.04	-0.23	-0.16	0.14	**-0.57****
	Increased temperature	0.27	-0.27	-0.23	-0.02	**0.27***	-0.29		**0.51***
PPT+	Duration	0.24	0.08	0.00	0.07	0.09	-0.34	0.29	
	Increased precipitation	**0.48***	0.04	0.26	**0.48***	**-0.53****	**-0.50***		0.50
PPT-	Duration	0.10	0.67	0.51	0.41	-0.07	**0.46***	0.26	0.27
	Decreased precipitation	0.42	-0.08	-0.01	0.21	**0.57****	-0.09	**0.55***	0.43
N	Duration	**-0.42****	**0.21****	0.03	**0.15***	0.10	-0.06	0.25	-0.27
	N rate	**-0.30****	-0.05	**0.23****	**0.24****	**0.50****	**-0.20****	0.12	0.06
P	Duration	-0.13	0.18	**0.33***	-0.12	0.18	-0.10		
	P rate	0.16	**0.46****	**0.41****	-0.17	-0.02	-0.02		
NPK	Duration	0.05	**0.46****	0.14	0.22	0.06	-0.11	0.29	
NP	Duration	-0.33	0.25	0.01	-0.37	-0.03	-0.22		

Table S9 Pearson's r correlation coefficients among GCFs induced shifts in edaphic factors and N transformation processes.

	ST	SM	PH	SOC	TN	NH_4^+	NO_3^-	DOC	AN	DON	MBC	MBN
PNR	**-0.44***	**0.17***	0.05	**0.31****	**0.20****	**0.20****	**0.29****	-0.11	0.31	0.17	0.01	**0.26***
DEA	0.46	0.04	**0.27***	0.20	0.11	-0.10	0.13	0.07	**0.66***		-0.33	0.60
N2O	0.22	0.19	**-0.37***	0.28	0.43	**0.43****	**0.27****				0.53	0.32
N min	**-0.58***	0.28				**0.52****	**0.48****		**0.51****	-0.16	0.58	0.37
NEA	**0.62***	0.18					-0.21					