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Effects of multiple global change factors on soil microbial richness, diversity and functional gene abundances: A meta-analysis

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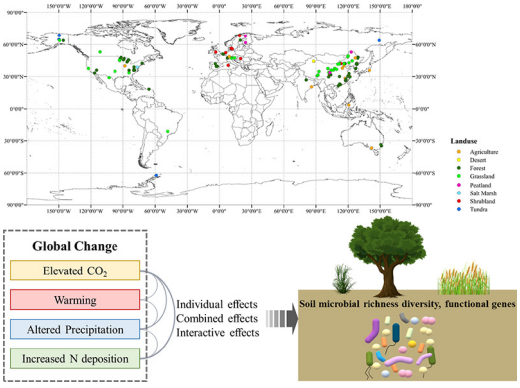
HIGHLIGHTS

- Global change effects on soil microbial properties were conducted by meta-analysis.
- eCO_2 increased microbial richness and diversity by 40.5% and 4.6%, respectively.
- Warming and N addition decreased denitrification functional gene abundances.
- N addition had larger effects on C-cycling functional genes than N-cycling ones.
- Additive interactions are found in most factor pairs, followed by synergy.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil microbial richness, diversity, and functional gene abundance are crucial factors affecting belowground ecosystem functions; however, there is still a lack of systematic understanding of their responses to global change. Here, we conducted a worldwide meta-analysis using 1071 observation data concerning the effects of global change factors (GCFs), including warming (W), increased precipitation (PPT+), decreased precipitation (PPT-), elevated CO_2 concentration (eCO_2), and nitrogen deposition (N), to evaluate their individual, combined, and interactive effects on soil microbial properties across different groups and ecosystems. Across the dataset, eCO_2 increased microbial richness and diversity by 40.5% and 4.6%, respectively; warming and N addition decreased the abundance of denitrification functional genes (*nirS*, *nirK*, and *nozS*); N addition had a greater impact on soil C-cycling functional genes than on N-cycling ones. Long-term precipitation change was conducive to the increase in soil microbial richness, and fungal richness change was more sensitive than bacterial richness, but the sensitivity of bacteria richness to N addition was positively correlated with experimental duration. Soil microbial richness, diversity, and functional gene abundances could be significantly affected by individual or multiple GCF changes, and their interactions are mainly additive. W × eCO_2 on microbial diversity, and N × PPT+ and W × N on N-cycling functional gene abundance showed synergistic interactions. Based on the limitations of the collected data and the findings, we suggest designing experiments with multiple GCFs and long experimental durations and incorporating the effects and interactions of multiple drivers into ecosystem models to accurately predict future soil microbial properties and functions under future global changes.

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1. Introduction

The earth is home to as many as 1 trillion (10^{12}) species of microbes (Locey and Lennon, 2016). Soil, with its special physical structure and complex chemical composition, is an ideal habitat for microbes and an environmental medium with the most abundant and diverse microorganisms (Jansson and Hofmockel, 2020). Soil may contain up to 1 billion (10^9) bacterial cells per gram, comprising thousands of taxa (Wagg et al., 2014). Soil microbial communities play crucial roles in almost all biogeochemical processes in terrestrial ecosystems, such as primary productivity, greenhouse gas emissions, nutrient circulation, and organic decomposition (Delgado-Baquerizo et al., 2017; Morrissey et al., 2019). Soil microbial communities (composition, diversity, or abundance) and their functionality are related to environmental factors, including soil physical and chemical properties, vegetation, and global changes (Gao et al., 2019).

The Earth is undergoing a host of global changes, such as elevated atmospheric CO_2 concentrations (eCO_2), warming (W), altered precipitation, and elevated atmospheric nitrogen (N) deposition (IPCC, 2014). Global changes factors (GCFs) affect ecosystem functions by regulating soil microbial communities (Johnston et al., 2019). The species may fall outside their climatic niches due to global change and could lead to loss of biodiversity or even species extinction (Bellard et al., 2012).

Soil microbes respond to global changes in various ways (Yang et al., 2021). Studies based on small-scale controlled experiments have shown that eCO_2 increases plant biomass by enhancing plant photosynthesis and increases soil carbon (C) input by litter and root exudates, providing nutrients for soil microbes (Brechet et al., 2018); eCO_2 can promote organic C decomposition, increase active C distribution, improve microbial activity, stimulate soil extracellular enzyme activity (Jansson and Hofmockel, 2020). However, Dunbar et al. (2012) reported no common effect of eCO_2 on bacterial biomass, richness or community composition across different ecosystems. Warming promotes soil microbial metabolism, increases enzyme activities, accelerates the decomposition of organic matter, induces nutrient utilization by plants and microorganisms, and is beneficial to plant growth and litter feedback to soil (Mouginot et al., 2014); conversely, warming may aggravate nutrient or water limitations of plants and microbes (Melillo et al., 2017). Altered precipitation affects soil water balance and soil aeration, with corresponding changes in microbes and nutrient cycling (Wang et al., 2014). Stovicek et al. (2017) found that soil microbial diversity was high under dry conditions because of the fragmentation of niches in dry soils, but drought may also reduce the genetic potential and stability of soil microbiomes (Neilson et al., 2017). N deposition increases the availability of soil nutrients and causes soil acidification. The increased vegetation net productivity and litter input to soil impacts soil element stoichiometry, nutrient utilization and limitation, thus changing soil microbial communities of different trophic types (Fierer et al., 2012). N deposition also influences functional genes and species related to N cycling (Nie et al., 2019). These GCFs (W, eCO_2, N, and altered precipitation) have been reported to intensely impact soil microbes, but their effects on different microbial groups and the contribution of the GCFs still need further research.

Global change involves the simultaneous occurrence of multiple factors, and the positive or negative influences of GCFs on microbial communities are driven directly and indirectly by other GCFs, which may offset or advance each other. Eisenhauer et al. (2012) conducted an experiment on grassland ecosystems with eCO_2, drought, and increased N deposition and found that soil microbial biomass increased with eCO_2, while there were interactions between every pair of GCFs. Warming accelerated soil respiration and nitrogen mineralization when there is no moisture limit, whereas in dry soil environments, it tended to decrease (Thakur et al., 2018). This subsequently intensified soil N restrictions, so increased N deposition can compensate for the N deficiency in dry environments (Melillo et al., 2011). However, under humid soil conditions, N addition may further aggravate the negative impacts on the growth of soil microorganisms (Reich et al., 2014). The interactions of GCFs cannot be neglected. Therefore, there is a prior significance of combined effects compared with the individual effects of multiple global drivers, which is more conducive to simulating the response of soil microorganisms under actual climate change scenarios (Yue et al., 2018).

Meta-analysis is a statistical approach that integrates the quantitative results of previously published studies and obtains a general trend with greater statistical power (Romero-Olivares et al., 2017). For example, meta-analyses have shown that GCFs influences soil respiration (Zhou et al., 2016), soil nutrients (Yue et al., 2019), enzyme activities (Meng et al., 2020), and microbial biomass (Ren et al., 2018). The lack of evaluation of the soil microbial diversity, richness, and functionality in response to multiple GCFs discourages predicting their future changes and functioning, and obstructs their incorporation into biogeochemical cycles and Earth system models under future scenarios with global changes. Therefore, a meta-analysis was conducted and synthesized from previously published articles to quantitatively assess the individual, combined, and interactive effects of GCFs (including eCO_2, warming, altered precipitation, and N addition) on soil microbial properties in different regions and ecosystems. The main issues addressed were (1) quantification of the effects of GCFs individually or in combination on soil microbial properties; (2) investigation of whether the interactions of the GCFs on soil microbial properties are additive, synergistic, or antagonistic; and (3) examination of whether environmental or experimental conditions influence the responses of soil microbial properties, and discussion of the potential mechanisms of soil microbial communities responding to global changes.

2. Methods and materials

2.1. Data collection

Soil bacterial and fungal richness, frequently reported by operational taxonomic units (OTUs), Chao and ACE indices, diversity (Shannon index), and C- and N-cycling functional genes (i.e., *mcrA*, *pmoA*, archaeal *amoA*, bacterial *amoA*, *nifH*, *nosZ, nirK*, and *nirS*) associated with GCFs were retrieved from the Web of Science and Chinese Science Citation Database (CSCD) until April 2021 (Zhou et al., 2020).

The keywords used were "soil" and "climate change" or "global change" or "CO_2 enrichment" or "elevated CO_2" or "elevated carbon dioxide" or "decreased precipitation" or "decreased rainfall" or "drought" or "altered precipitation" or "altered rainfall" or "increased rainfall" or "increased precipitation" or "water addition" or "warming" or "elevated temperature" or "increased temperature" or "N deposition" or "N fertilization" or "N addition" and "organism" or "microorganism" or "microbe" or "biota" or "biodiversity" or "bacteria" or "fungi" or "archaea" or "underground community" or "functional gene" or "OTU". To be included in the database, proper papers needed to satisfy the following criteria: (1) choosing field studies and excluding laboratory studies; (2) experiments containing at least one of the target soil microbial properties in response to at least one of the mentioned GCFs; (3) at least 2 × 2 full-factorial experiments were designed, and the control and experimental groups were carried out at the same sites or with the same raw soils; (4) target variables were measured under the same conditions for both control and experimental groups, and experimental conditions and treatments were clearly recorded; (5) experimental duration was more than one growing season or one year; (6) sample sizes, mean values, standard errors (SE), or standard deviations (SD) were recorded in tables, figures, or text; and (7) experimental data from only the topsoil layer (< 20 cm) in terrestrial ecosystems were included.

A total of 1071 observations (Supplementary Materials 1 Table S2 and Supplementary Materials 2 Dataset) were collected based on the above criteria following a PRISMA flow and checklist (Supplementary Materials 1 Fig. S1 and Table S1), and the publication list is shown in Supplementary Materials 1 Text S1. The global distribution of the experimental sites is shown in Fig. 1. In addition, the mean annual temperature (MAT), and mean annual precipitation (MAP), and experimental forcing factors (i.e., experimental duration and magnitude of controlled GCFs) were also recorded.

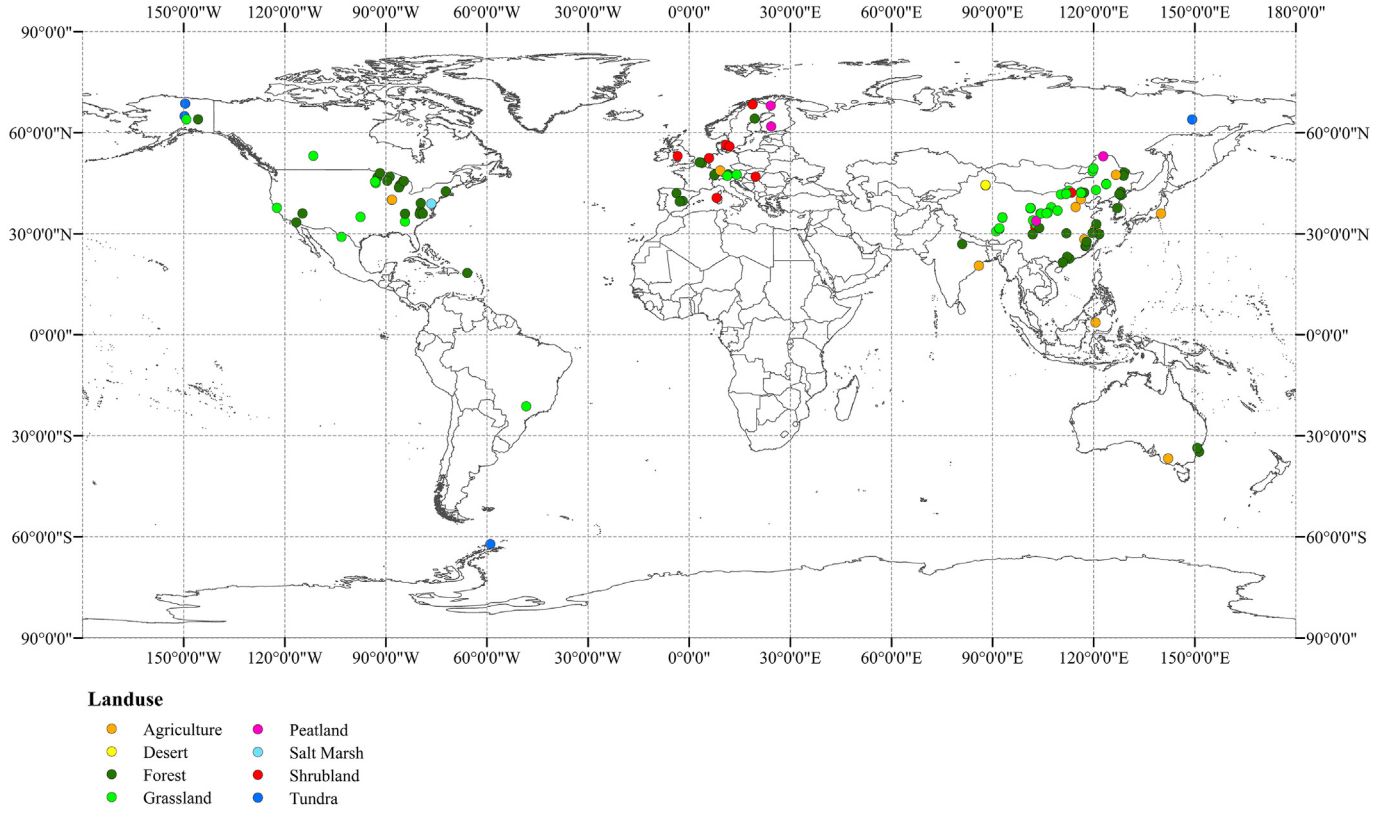


Fig. 1. Global distribution of the study sites among different ecosystems included in this meta-analysis.

2.2. Data analysis

2.2.1. Effects of global change factors

The effects of GCFs (both the effects of a single factor and the combined effects of multiple factors) were defined as the target variable responses compared with the control group (Crain et al., 2008). The response ratios (*RRs*) were defined as the ratio of the target soil microbial property mean values in the experimental group ($\overline{X_e}$) to those of the control group ($\overline{X_c}$) to evaluate the response of soil microbial properties to multiple GCFs. of the treatment with GCFs was changed to (Eq. (1)). The logarithm of the *RR* (ln*RR*) was used to ensure a normal sampling distribution and reduce bias (Hedges et al., 1999).

$$lnRR = ln\left(\frac{\overline{X_e}}{\overline{X_c}}\right) = ln\,\overline{X_e} - ln\,\overline{X_c} \tag{1}$$

where $\overline{X_c}$ and $\overline{X_e}$ are the mean value of a target variable in the control and experimental groups, respectively, and the positive or negative ln*RR* values indicate increases or decreases in the target valuables with the GCF changes, respectively. The corresponding variance of the ln*RR* (V_{lnRR}) was calculated based on the SD from each primary study.

$$V_{lnRR} = \frac{S_e^2}{n_e\overline{X_e}^2} + \frac{S_c^2}{n_c\overline{X_c}^2} \tag{2}$$

where S_c and S_e are the SD of a target variable in the control and experimental groups, respectively, and n_c and n_e are the sample sizes of a target variable in the control and experimental groups, respectively. If the variation was expressed as SE, then the SE was first converted to SD.

The overall effects of GCFs on soil microbial properties were determined using a weighted random-effect model (Hedges et al., 1999). The weighted response ratio ($\ln RR_{++}$) was calculated using Eq. (3).

$$lnRR_{++} = \frac{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij} lnRR_{ij}}{\sum_{i=1}^{m}\sum_{j=1}^{k} w_{ij}} \tag{3}$$

where m is the number of groups, k is the number in the ith group, w is the weight for each ln*RR*, and w was calculated as the reciprocal of the variance ($1/V_{\mathrm{lnRR}}$). If the 95% confidence interval (CI) did not include 0, $\ln RR_{++}$ was considered significantly different from 0.

2.2.2. Sensitivity

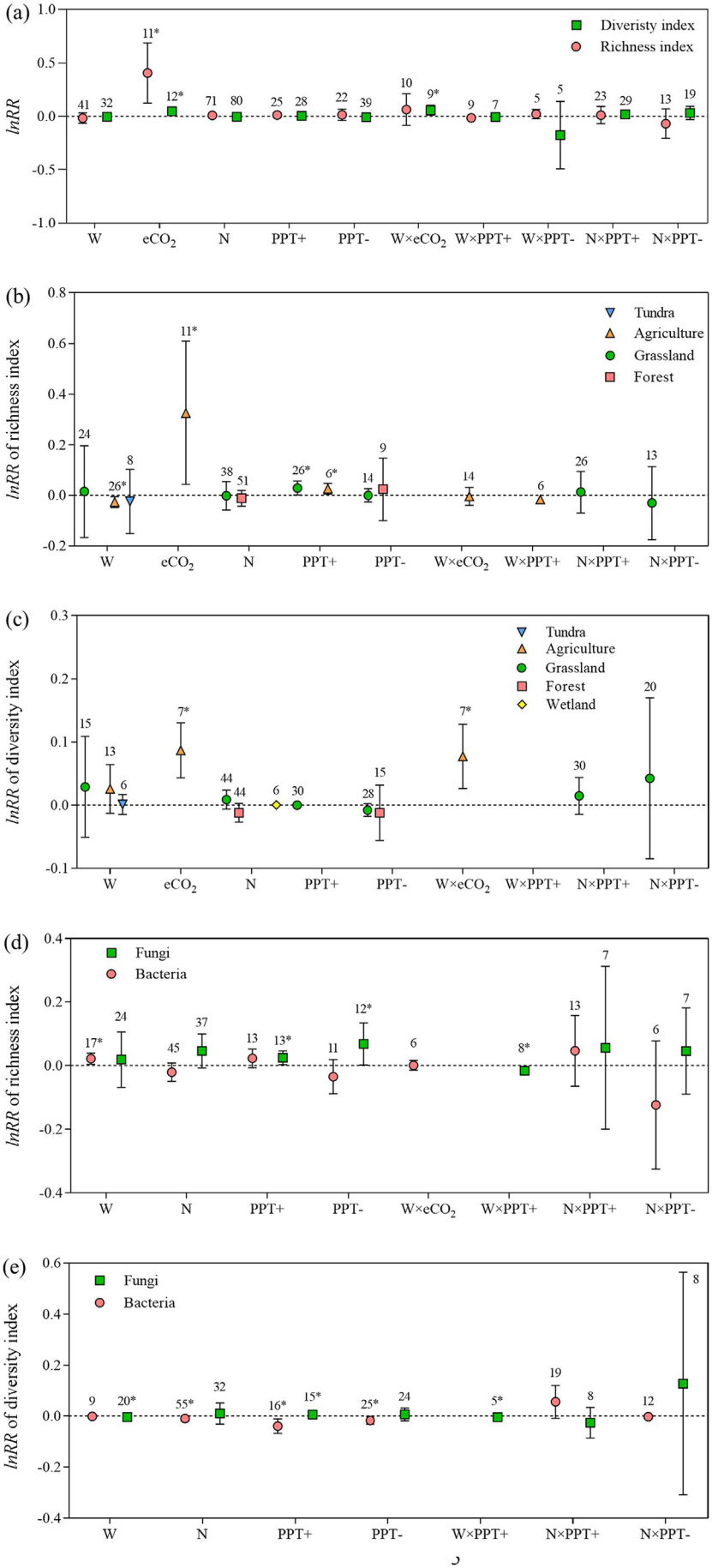
To achieve comparable the microbial responses to GCFs in different studies, we calculated the sensitivity to represent the response degree to global change (Zhou et al., 2018). This was used to explore the relationship between the response and environmental factors, and was calculated as follows:

$$Sensitivity = \frac{lnRR}{\Delta GCF} \tag{4}$$

where ΔGCF is the magnitude of the GCFs in the experimental groups compared to those in the control groups.

2.2.3. Main effects and interactions

If the combined effect of multiple factors was equal to the summation of individual effects or if the difference was not significant, we considered the interaction additive; if the combined effect was less or greater than the summation of individual effects, an antagonistic or synergistic interaction occurred (Crain et al., 2008). The main effect of a factor was calculated as the difference between the response value of the target variable in the presence and absence of another factor (Crain et al., 2008). The main effects and interactions were calculated using Hedges' *d*, which is an estimate of


(a)
lnRR
Diveristy index
Richness index
W
eCO2
N
PPT+
PPT-
W×eCO2
W×PPT+
W×PPT-
N×PPT+
N×PPT-
(b)
lnRR of richness index
Tundra
Agriculture
Grassland
Forest
(c)
lnRR of diversity index
Tundra
Agriculture
Grassland
Forest
Wetland
(d)
lnRR of richness index
Fungi
Bacteria
(e)
lnRR of diversity index
Fungi
Bacteria

the standardized mean differences and is not biased by small sample sizes (Yue et al., 2017). The main effects of GCFs A and B (d_A and d_B) and their interaction (d_I), as calculated using Eqs. (5)–(7).

$$d_A = \frac{(X_A - X_{AB}) - (X_B - X_C)}{2s} J(m) \tag{5}$$

$$d_B = \frac{(X_B + X_{AB}) - (X_A + X_C)}{2s} J(m) \tag{6}$$

$$d_I = \frac{(X_{AB} - X_A) - (X_B - X_C)}{2s} J(m) \tag{7}$$

where X_A, X_B, X_{AB}, and X_C are the mean values of a target variable in the experimental groups A and B, their combination (i.e., A × B) and in the control group, respectively; and s is the pooled SD, and $J(m)$ is the correction term for small sample bias.

$$s = \sqrt{\frac{(n_C - 1)S_C^2 + (n_A - 1)S_A^2 + (n_B - 1)S_B^2 + (n_{AB} - 1)S_{AB}^2}{n_C + n_A + n_B + n_{AB} - 4}} \tag{8}$$

$$J(m) = 1 - \frac{3}{4m - 1} \tag{9}$$

where n_A, n_B, n_{AB}, and n_C are the sample sizes, and S_A, S_B, S_{AB}, and S_C are the SD in the experimental groups of A, B, their combination (i.e., A × B) and in the control group respectively; and m is the degree of freedom: $n_C + n_A + n_B + n_{AB} - 4$. The variance of Hedges' d was calculated using Eq. (10):

$$V_{d_t} = \frac{1}{4}\left[\frac{1}{n_C} + \frac{1}{n_A} + \frac{1}{n_B} + \frac{1}{n_{AB}} + \frac{d_t^2}{2(n_C + n_A + n_B + n_{AB})}\right] \tag{10}$$

where t is the treatment of A, B, or A × B.

If the 95% CI overlapped with 0: (i) the main effect of an individual GCF was considered insignificant, whereas (ii) the interaction of the two GCFs was additive. In other cases (95% CI did not overlap with 0): if the GCF pairs whose individual effects were negative or in opposite directions, positive interaction effect sizes were considered to be antagonistic, and negative interaction effect sizes were considered to be synergistic; for pairs whose individual effects were both positive, positive interactive effects were considered to be synergistic interactions, and negative interactive effects were considered to be antagonistic. Small sample sizes (less than 5) may cause considerable uncertainty, and results with limited sample sizes are not shown.

2.3. Statistical analysis

GetData Graph Digitizer 2.26 (http://getdata-graph-digitizer.com/) was used to obtain the original data from graphs or figures from published papers. The database was set up in Excel 2016 (Microsoft, Seattle, WA, USA). The meta-analysis was performed using MetaWin 2.1 (Sinauer Associates Inc., Sunderland, MA, USA). Significance and regression analyses were performed using SPSS version 25.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Effects of global change factors on soil microbial communities

The global distribution of the experimental cases meeting the criteria in Fig. 1 shows that most studies were from China, the US and Europe. Pooling all soil microbial richness and diversity data across ecosystems and microbial groups (Fig. 2a), we found that GCFs did not always change microbial richness and diversity. The effect of eCO_2 on microbial richness was significantly positive (+33.0%), as was it on microbial diversity (+5.1%, $p < 0.05$); individual W treatment had a negative effect on microbial diversity (−0.4%, $p < 0.05$), and a positive effect was also observed with the combined W × eCO_2 treatment upon microbial diversity (+5.5%, $p < 0.05$). PPT+ significantly increased microbial richness (+2.6%, $p < 0.05$), and the combined treatment of W × PPT+ significantly reduced the richness (−1.4%, $p < 0.05$) and diversity (−0.6%, $p < 0.05$) of microbes (Fig. 2a). N addition, PPT-, and combined treatments W × PPT-, N × PPT-, and N × PPT+ did not result in significant shifts in soil microbial indices ($p > 0.05$). In addition, the *lnRR* values of richness were greater than those of the corresponding diversity (except for the W × PPT- group).

Considering different ecosystems, PPT+ and eCO_2 significantly increased soil microbial richness (+2.6% and +32.6%, respectively) in agricultural ecosystems, while W significantly decreased it (−2.7%), and PPT + also significantly increased microbial richness in grassland soil (+2.8%) ($p < 0.05$, Fig. 2b). For soil microbial diversity, eCO_2 and W × eCO_2 significantly increased it (+8.7% and +7.7%, respectively) in agricultural soil ($p < 0.05$), while for other GCFs or in other ecosystems, the diversity index did not change significantly (Fig. 2c). Overall, the results showed that agricultural soil microbes were more likely to be impacted by GCFs than other ecosystems (Fig. 2b and c).

The data were then divided into microbial groups; warming significantly increased bacterial richness (+2.1%) ($p < 0.05$, Fig. 2d). An interesting discovery was found in the treatment of altered precipitation: PPT- and PPT+ both increased fungal richness (+6.8% and + 2.4%, respectively) but did not significantly alter bacterial richness, whereas the W × PPT+ combined treatment decreased fungal richness by 1.7% ($p < 0.05$, Fig. 2d). Moreover, N addition and PPT- and PPT+ significantly decreased bacterial diversity (−1.0%, −1.8%, and −4.0%, respectively) ($p < 0.05$, Fig. 2e). Warming decreased fungal diversity by 0.4%, PPT+ increased it (+0.5%), and the combination of W × PPT+ showed a negative effect (−0.5%) on fungal diversity ($p < 0.05$, Fig. 2e).

The responses of soil functional gene abundances to GCFs are shown in Fig. 3. The abundances of C-cycling functional genes were significantly increased by eCO_2 (+49.1%), N deposition (+3.0%), combined W × eCO_2 (+39.6%), while decreased by combined W × PPT- (−66.5%) ($p < 0.05$, Fig. 3a); N-cycling functional gene abundances were significantly decreased by W (−14.2%), while increased by eCO_2 (+19.5%) and combined N × PPT+ (+31.8%) ($p < 0.05$, Fig. 3a); and individual changes of precipitation did not significantly affect abundances of both C- and N-cycling functional genes ($p > 0.05$, Fig. 3a).

For C-cycling functional genes, eCO_2 significantly increased the abundance of *mcrA* and *pmoA* by 33.7% and 41.8%, respectively ($p < 0.05$, Fig. 3b). Warming and PPT- had no significant effect on *mcrA* ($p > 0.05$) but significantly decreased the abundance of the *pmoA* gene (−13.4% and − 45.7%, respectively), and the combined W × PPT- treatment showed a significantly negative effect on *pmoA* abundance (−68.7%) ($p < 0.05$, Fig. 3b). W × PPT- also decreased *mcrA* abundance by 73.2%, while N addition increased it by 4.8% ($p < 0.05$, Fig. 3b).

Fig. 2. Responses of soil microbial richness and diversity to global change factors. (a) Response ratio (*lnRR*) of microbial richness and diversity. (b) *lnRR* of soil microbial richness across different ecosystems. (c) *lnRR* of soil microbial diversity across different ecosystems. (d) *lnRR* of richness across microbial groups. (e) *lnRR* of diversity across microbial groups. W: warming; eCO_2: elevated carbon dioxide concentration; N: nitrogen addition; PPT-: decreased precipitation; PPT+: increased precipitation; W × eCO_2: warming plus elevated carbon dioxide concentration; W × PPT+: warming plus increased precipitation; W × PPT-: warming plus decreased precipitation; N × PPT+: nitrogen addition plus increased precipitation; N × PPT-: nitrogen addition plus decreased precipitation. Error bars represent 95% confidence intervals. The effect was considered significant if the 95% CI of the effect size did not cover zero, and marked with "*". The sample size for each variable is shown next to the point. Results were not presented when sample size was lower than 5.

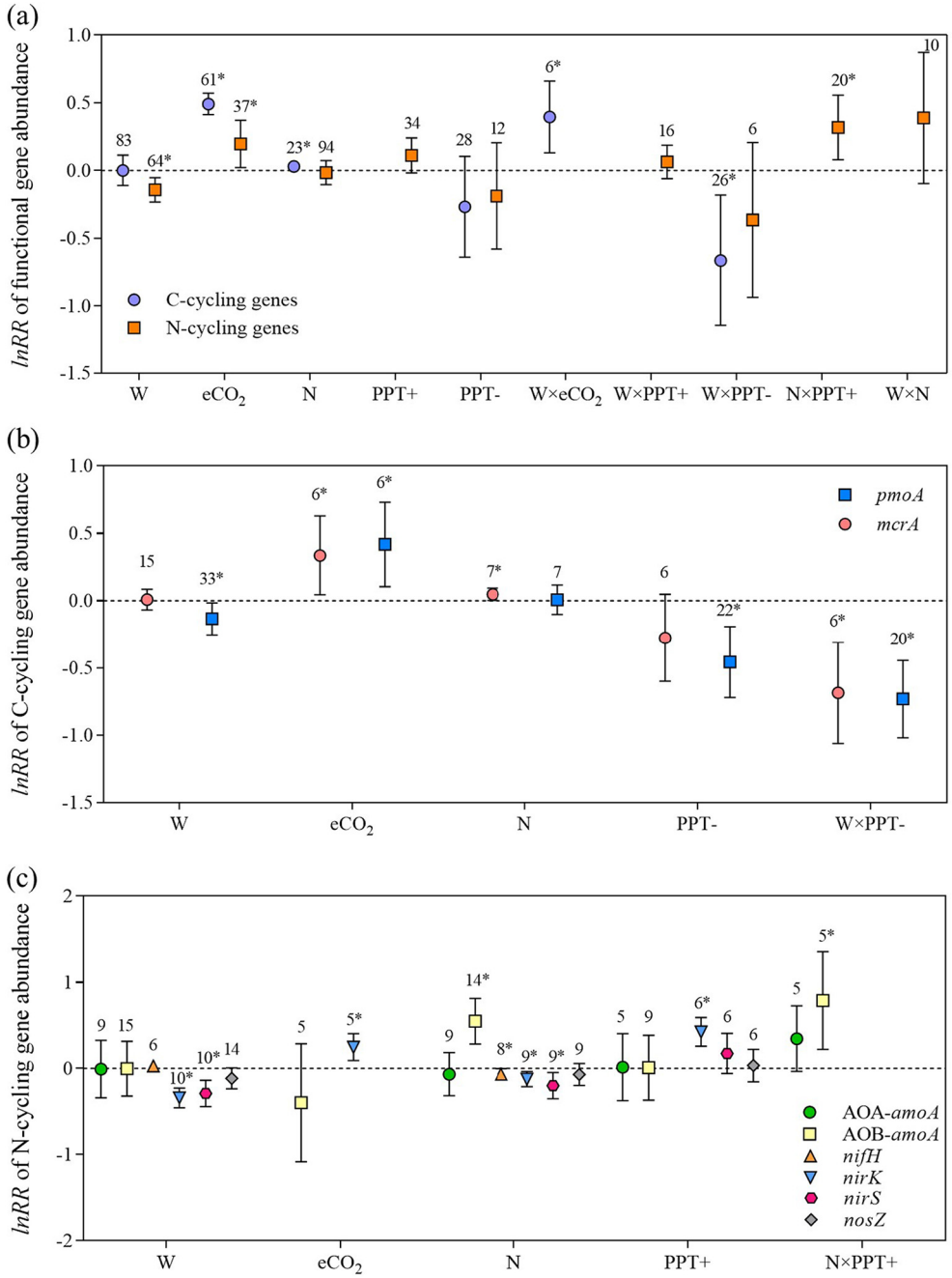


Fig. 3. Responses of soil functional gene abundance to global change factors. (a) Response ratio (*lnRR*) of soil C- and N-cycling gene abundance. (b) *lnRR* of soil C-cycling gene abundance. (c) *lnRR* of soil N-cycling gene abundance. W: warming; eCO_2: elevated carbon dioxide concentration; N: nitrogen addition; PPT-: decreased precipitation; PPT+: increased precipitation; W × eCO_2: warming plus elevated carbon dioxide concentration; W × PPT+: warming plus increased precipitation; W × PPT-: warming plus decreased precipitation; N × PPT+: nitrogen addition plus increased precipitation; N × PPT-: nitrogen addition plus decreased precipitation. Error bars represent 95% confidence intervals. The effect was considered significant if the 95% CI of the effect size did not cover zero, and marked with "*". The sample size for each variable is shown next to the point. Results were not presented when sample size was lower than 5.

Warming significantly decreased the abundance of denitrification functional genes (*nirS*: −29.1%; *nirK*: −34.2%) ($p < 0.05$), but did not significantly affect the abundance of AOA, AOB, *nifH*, and *nosZ* ($p > 0.05$, Fig. 3c). PPT+ and eCO_2 significantly increased the abundance of *nirK* by 42.4% and 24.7%, respectively ($p < 0.05$, Fig. 3c). However, the abundances of the *nifH*, *nirK*, and *nirS* genes were significantly decreased by N addition ($p < 0.05$) by 6.7%, 12.5%, and 20.1%, respectively (Fig. 3c). N addition and N × PPT+ significantly increased the abundance of *amoA*-AOB ($p < 0.05$), but the effects on *amoA*-AOA were not significant (Fig. 3c).

3.2. Sensitivities of soil microbial community responses to global change factors

The sensitivities of soil microbial richness, diversity and functional gene abundance to altered precipitation, N addition, and warming across different MAP, MAT, and experimental durations are shown in Figs. 4 and 5.

The sensitivity of bacterial diversity to altered precipitation was significantly negatively correlated with MAT ($p < 0.05$, Fig. 4d). The sensitivity of bacterial and fungal richness to altered precipitation was positively correlated with experimental duration ($p < 0.05$, Fig. 4g), but the relationship between bacterial richness sensitivity to N addition and experimental duration was

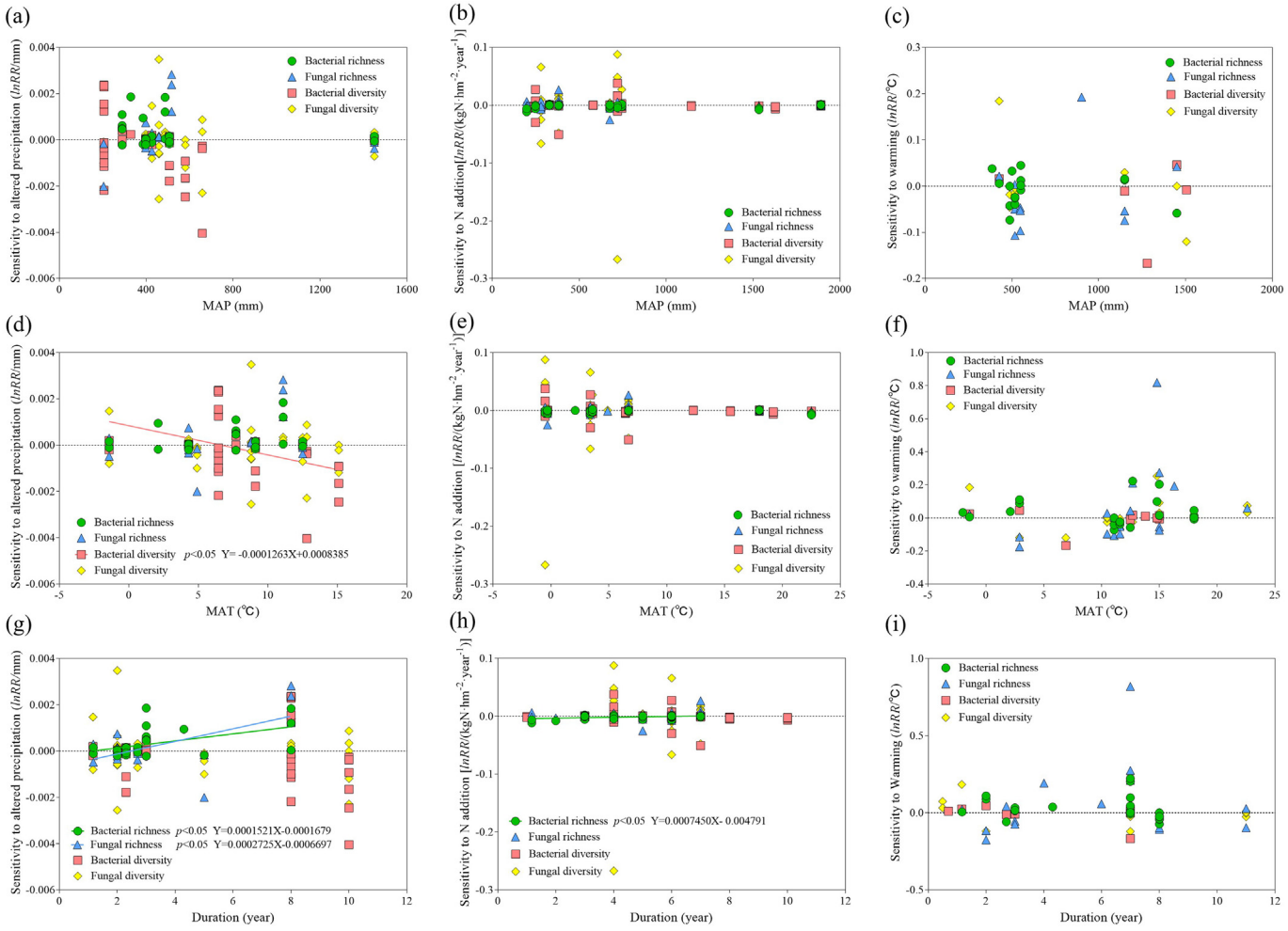


Fig. 4. Sensitivity of soil microbial richness and diversity to altered precipitation, N addition, and warming verses site-level MAP, MAT and experimental duration. (a) Sensitivity to altered precipitation (ln*RR*/mm) across different MAP. (b) Sensitivity to N addition [ln*RR*/(kgN·hm^{-2}·year^{-1})] across different MAP. (c) Sensitivity to warming (ln*RR*/°C) across different MAP. (d) Sensitivity to altered precipitation (ln*RR*/mm) across different MAT. (e) Sensitivity to N addition [ln*RR*/(kgN·hm^{-2}·year^{-1})] across different MAT. (f) Sensitivity to warming (ln*RR*/°C) across different MAT. (g) Sensitivity to altered precipitation (ln*RR*/mm) across different experimental duration. (h) Sensitivity to N addition [ln*RR*/(kgN·hm^{-2}·year^{-1})] across different experimental duration. (i) Sensitivity to warming (ln*RR*/°C) across different experimental duration. If the correlation is significant ($p < 0.05$), the linear equation is marked in the legend, and the linear fit line is shown in each sub-figure. Results were not presented when sample size was lower than 5.

positive ($p < 0.05$, Fig. 4h). N deposition was the most critical GCF that affected C- and N-cycling-related functional genes (Fig. 5). The sensitivity of *mcrA* gene abundance to N addition was significantly negatively correlated with MAP and MAT ($p < 0.05$, Fig. 5b and e). The sensitivity of *pmoA* to altered precipitation was significantly positively correlated with MAT ($p < 0.05$, Fig. 5d). The sensitivity of functional genes associated with denitrification (*nirS* and *nirK*) to N addition showed a significant positive relationship with MAP, MAT, and duration ($p < 0.05$, Fig. 5b, e, and h), and *nirS* sensitivity to warming was negatively correlated with MAT ($p < 0.05$, Fig. 5f).

3.3. Main and interactive effects of global change factors on soil microbial communities

The main effects and interactions of multiple GCFs on soil microbial communities are shown in Fig. 6. The main effects of W were significantly negative in W × eCO_2 and W × PPT + on soil microbial richness ($p < 0.05$, Fig. 6a-b), whereas W in W × PPT- on soil microbial richness and in W × eCO_2, W × PPT +, and W × PPT- on soil microbial diversity were also negative, but the main effects of W were not significant ($p > 0.05$, Fig. 6c, e-g). eCO_2 significantly induced positive main effects in W × eCO_2 on both microbial richness and diversity ($p < 0.05$, Fig. 6a and e). The main effects of PPT + were significantly positive in W × PPT + and N × PPT + on richness, and the main effect of PPT- in N × PPT- was also significantly positive ($p < 0.05$, Fig. 6b, d and i). N addition showed neutral main effects on both microbial richness and diversity. For functional genes, eCO_2 showed significantly positive main effects in W × eCO_2 on C-cycling functional gene abundance ($p < 0.05$, Fig. 6j), while W in W × PPT- showed significantly negative effects on C-cycling functional gene abundance ($p < 0.05$, Fig. 6k). PPT + showed significantly positive effects on N-cycling functional gene abundance in N × PPT + and W × PPT +, as well as W in W × N ($p < 0.05$, Fig. 6l, m, and o), while W in W × PPT + showed significantly negative effects ($p < 0.05$, Fig. 6o).

Additive interactions were dominant in all two-factor pair groups on soil microbial richness, diversity and functional gene abundances (Fig. 6). W × eCO_2 on microbial diversity, and N × PPT + and W × N on N-cycling functional gene abundance exhibited synergistic interactions (Fig. 6e, l, and m).

4. Discussion

4.1. Individual effects of global change factors on soil microbial communities

4.1.1. Effects of eCO_2 concentrations on soil microbial communities

The CO_2 concentration in the atmosphere is predicted to increase to 450–600 ppm by 2050 (IPCC, 2014). eCO_2 enhanced both the richness

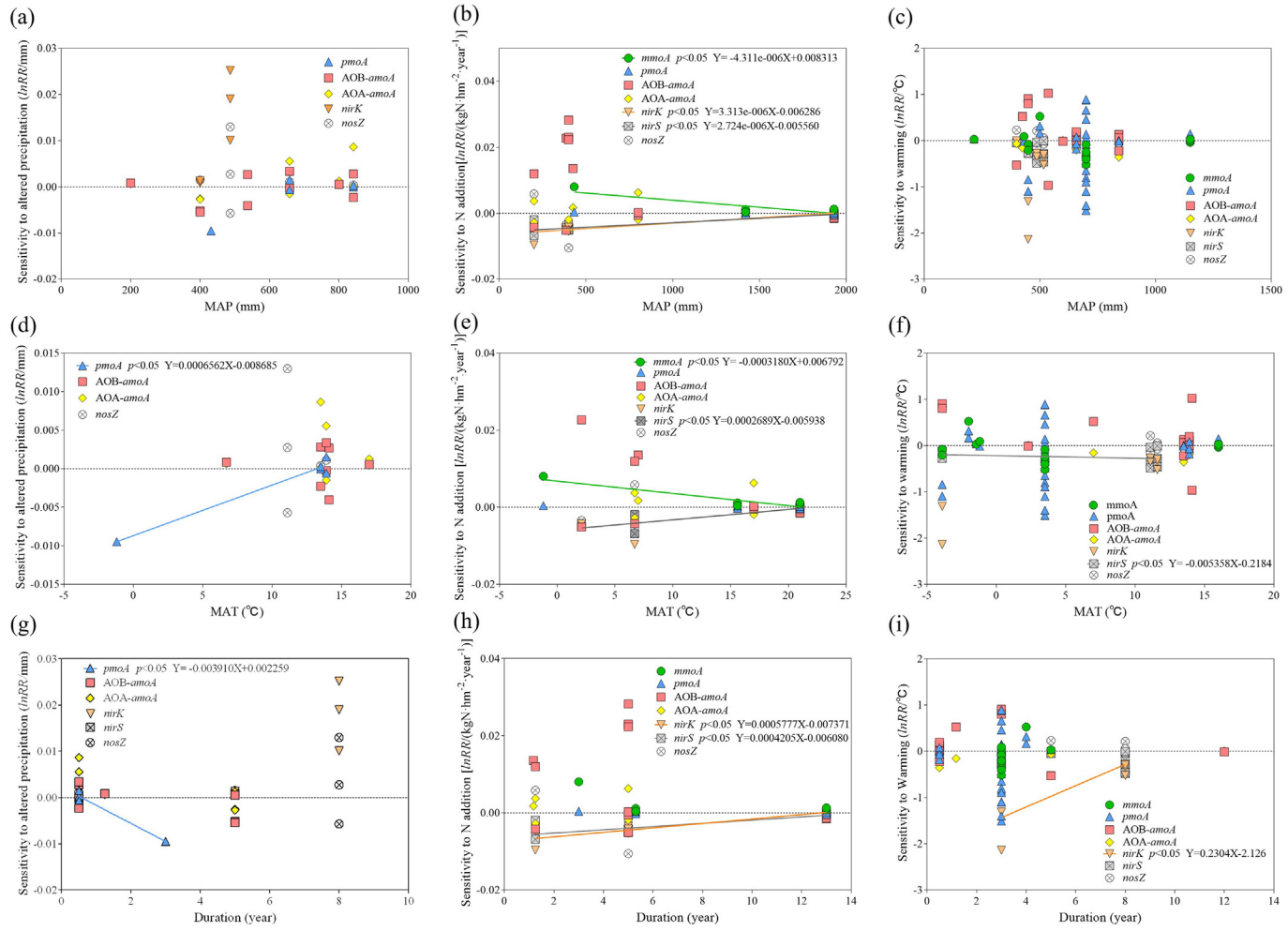


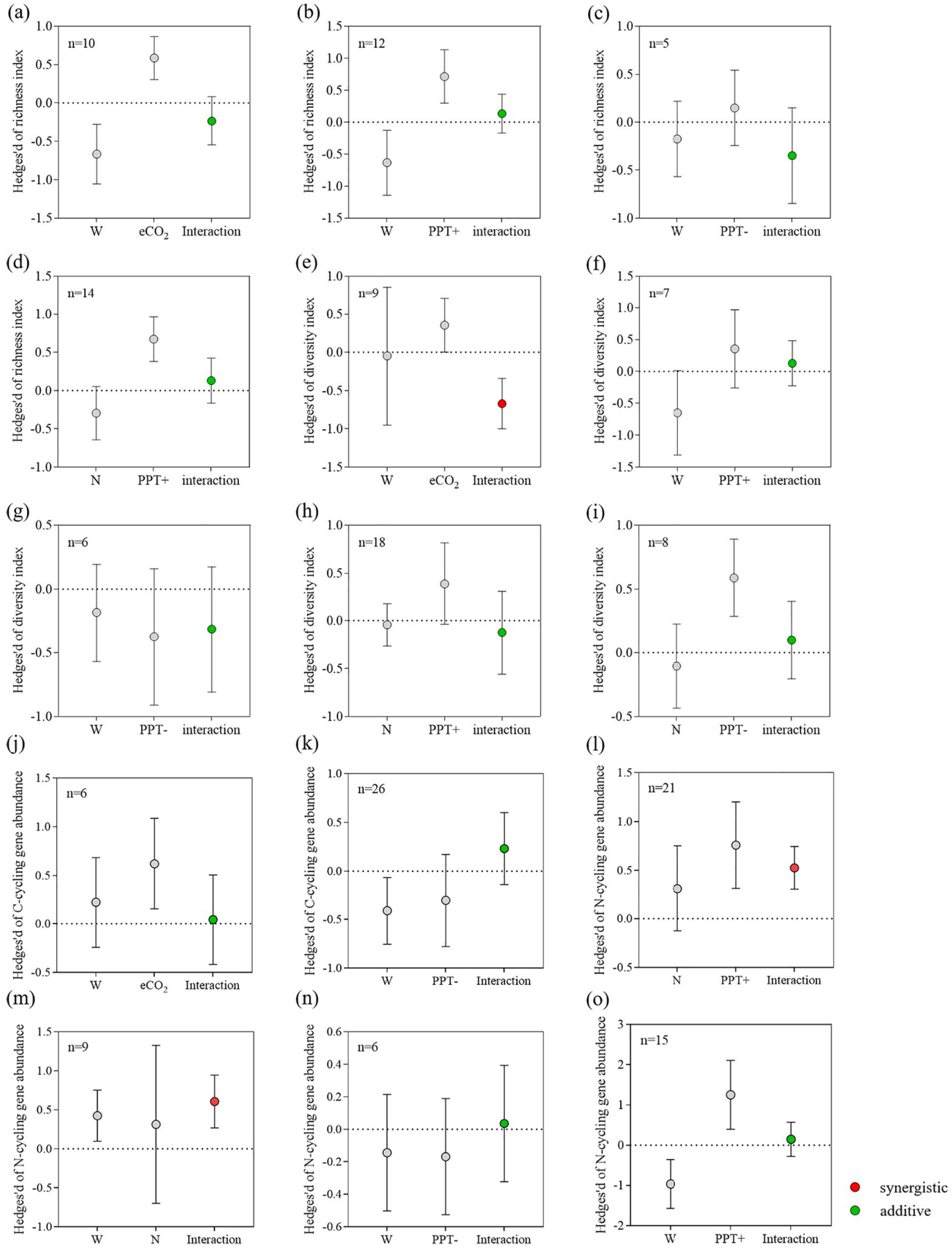
Fig. 5. Sensitivity of soil functional gene abundances to altered precipitation, N addition, and warming verses site-level MAP, MAT and experimental duration. (a) Sensitivity of soil functional gene abundances to altered precipitation (ln*RR*/mm) across different MAP. (b) Sensitivity of soil functional gene abundances to N addition [ln*RR*/(kgN·hm^{-2}·year^{-1})] across different MAP. (c) Sensitivity of soil functional gene abundances to warming (ln*RR*/°C) across different MAP. (d) Sensitivity of soil functional gene abundances to altered precipitation (ln*RR*/mm) across different MAT. (e) Sensitivity of soil functional gene abundances to N addition [ln*RR*/(kgN·hm^{-2}·year^{-1})] across different MAT. (f) Sensitivity of soil functional gene abundances to warming (ln*RR*/°C) across different MAT. (g) Sensitivity of soil functional gene abundances to altered precipitation (ln*RR*/mm) across different experimental duration. (h) Sensitivity of soil functional gene abundances to N addition [ln*RR*/(kgN·hm^{-2}·year^{-1})] across different experimental duration. (i) Sensitivity of soil functional gene abundances to warming (ln*RR*/°C) across different experimental duration. If the correlation is significant ($p < 0.05$), the linear equation is marked in the legend, and the linear fit line is shown in each sub-figure. Results were not presented when sample size was lower than 5.

and diversity of soil microbes (Fig. 2) and C- and N-cycling functional gene abundances (Fig. 3) according to this meta-analysis. The effects of eCO_2 on soil microbes are more likely to occur through enhanced photosynthetic C production and litter input to soil, as well as root exudation and nutrient availability (Sulman et al., 2014; Usyskin-Tonne et al., 2020). These changes lead to concomitant increases in SOC turnover and soil microbial respiration (Liang et al., 2017). In addition, eCO_2 is beneficial for SOC accumulation and provides resources for microbial growth (Hu et al., 2014). The functional genes *mcrA* and *pmoA* represent methanogens and methanotrophs, which are vital microorganisms responsible for the CH_4 cycle (Aronson et al., 2013; Peltoniemi et al., 2016). Methanogens are generally strict anaerobes, whereas methanotrophs can anaerobically oxidize CH_4 under anaerobic or anoxic conditions, control CH_4 release, and affect the balance of CH_4 and CO_2 production (Segarra et al., 2015; Jiang et al., 2020). The positive effects of eCO_2 on *mcrA* and *pmoA* may be due to increased soil C input, including root deposition and root exudates (Tokida et al., 2011; Wang et al., 2021). Denitrification was also demonstrated to be positive for eCO_2, which is likely due to the enhancement of soil microbial activity and N requirements by eCO_2 (Yu et al., 2018; Qiu et al., 2019).

4.1.2. Effects of warming on soil microbial communities

It has been reported that the Earth's surface temperature will increase by 1.0 to 3.7 °C by the end of the 21st century (IPCC, 2014). Most ecological models predict that warming will increase soil enzyme activity rates and soil respiration, accelerate the decomposition of organic matter and nutrient turnover, and increase plant growth and litter feedback to the soil (Mouginot et al., 2014). In this meta-analysis, warming increased the fungal richness and bacterial diversity. Warming can decrease microbial diversity but increase its population. Warming-induced decomposition of organic matter and enhanced nutrient availability for microbial growth (Guo et al., 2018; Guo et al., 2019). Warming decreased N-cycling functional genes, especially denitrification functional genes (*nirS* and *nirK*). The

Fig. 6. Main effects and interaction types in two-factorial designs of GCFs on soil microbial richness (a-d), diversity (e-i) and functional gene abundance (j-o). W: warming; eCO_2: elevated carbon dioxide concentration; N: nitrogen addition; PPT-: decreased precipitation; PPT+: increased precipitation. "n" in each sub-figure is the number of cases. Results were not presented when sample size was lower than 5.


(a)
n=10
Hedges'd of richness index
W
eCO2
Interaction
(b)
n=12
Hedges'd of richness index
W
PPT+
interaction
(c)
n=5
Hedges'd of richness index
W
PPT-
interaction
(d)
n=14
Hedges'd of richness index
N
PPT+
interaction
(e)
n=9
Hedges'd of diversity index
W
eCO2
Interaction
(f)
n=7
Hedges'd of diversity index
W
PPT+
interaction
(g)
n=6
Hedges'd of diversity index
W
PPT-
interaction
(h)
n=18
Hedges'd of diversity index
N
PPT+
interaction
(i)
n=8
Hedges'd of diversity index
N
PPT-
interaction
(j)
n=6
Hedges'd of C-cycling gene abundance
W
eCO2
Interaction
(k)
n=26
Hedges'd of C-cycling gene abundance
W
PPT-
Interaction
(l)
n=21
Hedges'd of N-cycling gene abundance
N
PPT+
Interaction
(m)
n=9
Hedges'd of N-cycling gene abundance
W
N
Interaction
(n)
n=6
Hedges'd of N-cycling gene abundance
W
PPT-
Interaction
(o)
n=15
Hedges'd of N-cycling gene abundance
W
PPT+
Interaction
synergistic
additive

reason for this might be that warming dried soils and denitrifiers are inhibited by aerobic conditions in drier soils, leading to a decrease in denitrifier abundance (Waghmode et al., 2018). The fact that PPT + increased the abundance of *nirK* confirmed this view (Fig. 3c). The warming-induced soil moisture decrease may also be a potential mechanism for the negative influence of warming on *pmoA*.

4.1.3. Effects of altered precipitation on soil microbial communities

In this meta-analysis, the phenomenon in which both PPT + and PPT- increased fungal richness may be because fungi are more resistant to water stress than bacteria as fungi have a filamentous structure and the ability to create large hyphal networks to accumulate osmoregulatory solutes and to protect their metabolism (Maestre et al., 2015; Rodriguez-Caballero et al., 2018). In addition, PPT + and PPT- both decreased bacterial diversity, and PPT + increased fungal diversity. Altered precipitation can change soil water availability and shape plant community composition and productivity, thereby directly or indirectly shifting the abundance and composition of soil microorganisms (Ren et al., 2018). Decreased precipitation is unfavorable for methanogens and methanotrophs in anaerobic environments (Wu et al., 2020), and warming-induced soil moisture decreases superimposed with PPT- may be a potential mechanism for the negative influence on *mcrA* and *pmoA*.

4.1.4. Effects of increased N deposition on soil microbial communities

N deposition increases soil N availability, intensifies soil acidification, and directly or indirectly affects soil microbial richness and composition (Li et al., 2019; Yang et al., 2020). More N input to soil may have toxic effects on some microorganisms (Li et al., 2019) and may increase competition for non-N nutrients and ultimately decrease bacterial diversity (Fig. 2e) (Zhou et al., 2017). N addition could increase N availability and promote the activity of methanogenic archaea (Kong et al., 2019; Wu et al., 2020), thus increasing the abundance of *mcrA* (Fig. 3b). This result implied that *amoA*-AOB were more responsive to the availability of the N substrate than *amoA*-AOA. This might be because *amoA*-AOA and *amoA*-AOB have different ecological niches (Martens-Habbena et al., 2009). *amoA*-AOB has a higher level of ribosomal content and may be more adaptable in a nutrient-rich environment, and N addition in moderation improves its competitiveness (Li et al., 2020a). The decrease in the N-fixing gene *nifH* may be because N addition leads to more available N in soil; thus, no extra metabolic investment is required based on the principle of resource acquisition (Li et al., 2020a). Soil acidification and the high salinity caused by excessive mineral N application may cause a decrease in denitrifiers (Tang et al., 2016).

It has been reported that N deposition has a more significant impact on soil C-cycling functional genes than N-cycling ones. The influence of N addition on C cycling may be due to plant litter quality, the extent of litter decay, litter decomposition rates, and microbial communities (Whittinghill et al., 2012; Garcia-Palacios et al., 2015). However, different types of functional genes associated with N cycling, including denitrification, ammonia oxidation, and N fixation, showed different patterns of responses to N addition, and the insignificant impacts on the overall N-cycling functional genes may be due to the offsets of different functional genes (Fig. 3c).

4.2. Combined and interactive effects of global change factors on soil microbial communities

The interaction between two factors includes addition, synergism, and antagonism (Crain et al., 2008). In this meta-analysis, the effects of two pairs of GCFs on soil microbial richness, diversity, and functional gene abundance and additive interactions were also dominant. The interaction of $W \times eCO_2$ may occur in all the three forms. For example, warming-accelerated nutrient mineralization could offset CO_2-induced nutrient limitations and result in a CO_2 fertilization effect (Dieleman et al., 2012). Similarly, the increased water use efficiency due to eCO_2 could overcome water restrictions due to warming (Morgan et al., 2011). Hence, it is possible that $W \times eCO_2$ factors present a synergistic interaction on soil microbial diversity, and that the combination treatment had significantly positive effects on soil microbial diversity in this meta-analysis (Fig. 2a).

The results shown in Fig. 6 imply that the N effects of N on N-cycling functional genes depend on temperature or precipitation fluctuations, and the results are consistent with those of Li et al. (2020b). For microorganisms, water may be a limiting factor prior to N. Most microbial functional groups respond to N addition positively only in conditions without water stress (Zhang et al., 2015). The added N enters the soil solution effectively if soil moisture is sufficient, thus increasing available N for microbes and accelerating the activity of related genes and enzymes (Wang et al., 2014). This could explain why the combined effects of $N \times PPT +$ on N-cycling functional gene abundances were significantly positive (Fig. 3a), PPT + induced a main effect, and the interaction of $N \times PPT +$ was synergistic (Fig. 6l). The transformation and circulation of N in soils are closely related to soil C resources and are regulated by microbes (Geisseler et al., 2010). Warming can affect soil bacterial communities directly via metabolic carbon (Schindlbacher et al., 2011) and can affect organic matter decomposition rates and thus potentially regulate soil bacterial communities indirectly via the soil nutrient pool (Ma et al., 2018). Therefore, we found a main effect of W on N-cycling functional genes and synergistic interactions between $W \times N$.

4.3. Sensitivity of soil microbial richness, diversity and functional gene abundance responses to GCFs

Sensitivity of bacterial diversity to altered precipitation implied that the sensitivity to PPT was higher at cold and hot sites than at sites with moderate temperatures (close to 0). A previous study also reported a greater variation in the soil bacterial Shannon index in temperate forests than in tropical/subtropical forests (Zhou et al., 2020). Temperate soils may have a stable soil bacterial diversity. Long-term precipitation change is conducive to an increase in soil microbial richness, and fungal richness change is more sensitive than bacterial richness. A short time after N addition, there was a significant decrease in soil bacterial richness (far from 0). This may be because N-induced soil property changes and nutrient constraints impacted the microbial community in long-term experiments (Zhou et al., 2016).

C- and N-cycling-related functional genes were more affected by N addition than the other GCFs. The effects of GCFs on ecosystem processes will change over time, so the experimental duration may be crucial for evaluating the responses of C- and N-cycling genes to GCFs (Li et al., 2020b). Higher temperature sensitivity of microbial activity has been reported in short-term studies (Dai et al., 2020). The negative relationship between the sensitivity of *mcrA* gene abundance to N addition and MAP or MAT implied that *mcrA* gene abundance was less sensitive to N addition at hot and wet sites (close to 0) than at cold and dry sites. Denitrification-related gene (*nirS* and *nirK*) sensitivity to N addition positively correlated with MAP, MAT, and experimental duration, and *nirS* sensitivity to warming was negatively correlated with MAT. The reason for these results may be that the cold and dry sites included mainly N-depleted grasslands, and N addition resulted in considerable nutrient changes (Tian et al., 2016).

In summary, understanding the effects of global change on soil microbial communities and functionalities and revealing their key mechanisms will help to improve predictions of ecosystem dynamics. Our meta-analysis verified that soil microbial communities are affected by global change and provide potential information for the development and testing of earth biogeochemistry models and further predicting the underlying consequences for terrestrial ecosystems. This global synthesis reported that intense human activity, different land types, regional climate specificity, and multiple GCF interactions should be incorporated into ecosystem models for accurate prediction and policy-making decisions to cope with global change.

5. Conclusions

The responses of soil microbial communities to global changes are crucial for ecosystem functions, but have not been studied. This meta-analysis focused on the individual, combined and interactive effects of multiple GCFs on soil microbial richness, diversity and functional gene abundance. We conclude that eCO_2 increased microbial richness and diversity; both PPT + and PPT- increased fungal richness, but W × PPT + decreased, and N addition and PPT + decreased bacterial diversity. Denitrification functional gene abundances were decreased by W and N. Agricultural soil microbes were more likely to be affected by GCFs than those in other ecosystems. MAT, MAP, and experimental duration significantly affected the soil microbial community response sensitivities to GCFs. Moreover, additive interactions were dominant for the two-factor pair GCFs, followed by synergistic interactions (W × eCO_2 on microbial diversity and N × PPT + and W × N on N-cycling functional gene abundance). Overall, this study can improve our understanding of the changes and responses in soil microbial communities and functions under global change and is important for ecosystem function prediction and policy-making under future global change regimes.

CRediT authorship contribution statement

Yuqian Li: Writing – original draft, Writing – review & editing, Conceptualization, Formal analysis. **Junwei Ma:** Resources, Conceptualization, Methodology. **Yi Yu:** Resources, Conceptualization, Methodology. **Yijia Li:** Writing – review & editing. **Xinyi Shen:** Writing – review & editing. **Shouliang Huo:** Project administration. **Xinghui Xia:** Funding acquisition, Project administration.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.152737.

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