Nitrification, denitrification, and related functional genes under elevated CO2: A meta-analysis in terrestrial ecosystems

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Received: 26 July 2022 | Revised: 7 November 2022 | Accepted: 7 December 2022

DOI: 10.1111/gcb.16568

Global Change Biology WILEY

RESEARCH ARTICLE

Nitrification, denitrification, and related functional genes under elevated CO_2: A meta-analysis in terrestrial ecosystems

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Abstract

Global change may have profound effects on soil nitrogen (N) cycling that can induce positive feedback to climate change through increased nitrous oxide (N_2O) emissions mediated by nitrification and denitrification. We conducted a meta-analysis of the effects of elevated CO_2 on nitrification and denitrification based on 879 observations from 58 publications and 46 independent elevated CO_2 experiments in terrestrial ecosystems. We investigated the effects of elevated CO_2 alone or combined with elevated temperature, increased precipitation, drought, and N addition. We assessed the response to elevated CO_2 of gross and potential nitrification, potential denitrification, and abundances of related functional genes (archaeal *amoA*, bacterial *amoA*, *nirK*, *nirS*, and *nosZ*). Elevated CO_2 increased potential nitrification (+28%) and the abundance of bacterial *amoA* functional gene (+62%) in cropland ecosystems. Elevated CO_2 increased potential denitrification when combined with N addition and higher precipitation (+116%). Elevated CO_2 also increased the abundance of *nirK* (+25%) and *nirS* (+27%) functional genes in terrestrial ecosystems and of *nosZ* (+32%) functional gene in cropland ecosystems. The increase in the abundance of *nosZ* under elevated CO_2 was larger at elevated temperature and high N (+62%). Four out of 14 two-way interactions tested between elevated CO_2 and elevated temperature, elevated CO_2 and increased precipitation, and elevated CO_2 and N addition were marginally significant and mostly synergistic. The effects of elevated CO_2 on potential nitrification and abundances of bacterial *amoA* and *nirS* functional genes increased with mean annual temperature and mean annual precipitation. Our meta-analysis thus suggests that warming and increased precipitation in large areas of the world could reinforce positive responses of nitrification and denitrification to elevated CO_2 and urges the need for more investigations in the tropical zone and on interactive effects among multiple global change factors, as we may largely underestimate the effects of global change on soil N_2O emissions.

KEYWORDS
climate change, drought, elevated CO_2, elevated temperature, increased precipitation, interactions, N_2O emissions, nitrogen addition, soil nitrogen cycling

1 | INTRODUCTION

Nitrogen (N) plays a pivotal role in terrestrial ecosystems, as N limits its primary productivity in many terrestrial ecosystems and thus the terrestrial carbon (C) sink (LeBauer & Treseder, 2008; Vitousek & Howarth, 1991). Among the microbially-mediated N cycling processes, nitrification (the oxidation of ammonium (NH_4^+) to nitrate (NO_3^-)) and denitrification (the reduction of NO_3^- into N-containing gases) are critical to the functioning of terrestrial ecosystems. Indeed, both nitrification and denitrification regulate mineral N availability in soils and contribute to ecosystem N losses through the production of NO_3^- that is prone to leaching and of N-containing gases, including nitrous oxide (N_2O), a potent greenhouse gas (Bateman & Baggs, 2005; Maag & Vinther, 1996). Nitrification and denitrification are the most important sources of N_2O emissions in soils, with N_2O being produced as a by-product of the first step of nitrification and as an intermediate or end-product during nitrifier denitrification and denitrification (Wrage et al., 2001, 2004). Assessing and understanding the impacts of global change on nitrification and denitrification are thus important, as the effects of global change on these microbial processes could induce positive feedback on climate change (Tian et al., 2020).

Numerous global change experiments have been conducted on soil N cycling in terrestrial ecosystems and have reported significant changes in nitrification and denitrification rates in response to global change (Barnard et al., 2005). These effects were mostly attributed to changes in the soil parameters to which nitrifying and denitrifying microbial communities are sensitive, such as soil moisture, soil oxygen content, soil labile C content, and soil mineral N content (Prosser, 1990; Tiedje, 1988). In particular, rising atmospheric CO_2 can induce significant changes in nitrification and denitrification rates through three main mechanisms. First, elevated CO_2 has been shown to increase soil moisture through decreased plant evapotranspiration (Field et al., 1995) and consequently to decrease soil oxygen content with differential effects on nitrifying and denitrifying microbial communities that are respectively mostly aerobic and anaerobic. Second, elevated CO_2 has been found to increase soil labile C content through increased net primary productivity and rhizodeposition (Hungate, 1999) with expected positive effects on denitrifying microbial communities that are heterotrophic for C. Third, elevated CO_2 has been reported to alter soil mineral N availability through its effect on the balance of N mineralization and immobilization and on plant and microbial N uptake (de Graaff et al., 2006; Kuzyakov et al., 2019) with cascading effects on nitrification and denitrification microbial processes that rely on NH_4^+ and NO_3^- availability.

Among the elevated CO_2 experiments conducted to date, divergent responses of nitrification and denitrification rates have been reported, with either negative, positive or neutral effects of elevated CO_2 on nitrification and denitrification rates (on nitrification, see Barnard et al., 2006 for decreases, Shen et al., 2021 or Waqas et al., 2021 for increases and Barnard et al., 2004 or Niboyet, Le Roux, et al., 2011 for no changes; on denitrification, see Barnard et al., 2004 for decreases, Baggs et al., 2003 or Carnol et al., 2002 for increases and Niboyet et al., 2010 or Niboyet, Le Roux, et al., 2011 for no changes). The responses to elevated CO_2 of the abundances of nitrifying and denitrifying functional genes, used as markers for the abundances of the nitrifying and denitrifying microbial communities in soils, have also been investigated: archaeal *amoA* gene for ammonia-oxidizing archaea (AOA), bacterial *amoA* gene for ammonia-oxidizing bacteria (AOB) (Leininger et al., 2006), *nirK* and *nirS* genes for denitrifying microbial communities that reduce nitrite (NO_2^-) to nitric oxide (NO), and *nosZ* gene for denitrifying microbial communities that reduce N_2O to N_2 (Kuypers et al., 2018; Levy-Booth et al., 2014). Similarly, discrepancies have been reported in the responses to elevated CO_2 of the abundances of nitrifying and denitrifying functional genes, with either decreases, increases or no significant changes in the abundances of archaeal *amoA*, bacterial *amoA*, *nirK*, *nirS* and *nosZ* functional genes in response to elevated CO_2 (on nitrifying functional genes, see Yang et al., 2019 for decreases, Shen et al., 2021 for increases and Nelson et al., 2010 or Simonin et al., 2015 for no changes; on denitrifying functional genes, see Kelly et al., 2013 for increases and Brenzinger et al., 2017 or Yang et al., 2019 for no changes).

This great inter-study variability of responses of nitrification and denitrification rates and abundances of related functional genes to elevated CO_2 clearly stresses the need to conduct a meta-analysis to assess the response of nitrification and denitrification to elevated CO_2. Divergent responses to elevated CO_2 among studies might notably be due to differences in ecosystem type, climate and soil characteristics, or magnitude and duration of the elevated CO_2 treatment–potential explanatory factors that a meta-analysis could uncover. Previous reviews and meta-analyses have been conducted to assess the effects of elevated CO_2 on nitrification and denitrification rates (Barnard et al., 2005; Liang et al., 2016) and abundances of nitrifying and denitrifying functional genes (Du et al., 2022; Li, Ma, et al., 2022), however, none of them have simultaneously analyzed the effects of elevated CO_2 on both rates and genes. Besides, global change is multifactorial, and these meta-analyses have not statistically assessed the effects of elevated CO_2 in combination with multiple other global change factors, including warming, increased precipitation, drought, and N addition. All these global change factors can also alter the soil conditions to which nitrifying and denitrifying microbial communities are sensitive and have been reported to elicit significant changes in nitrification and denitrification (Bai et al., 2013; Dai et al., 2020; Deng et al., 2021; Li, Zheng, et al., 2020; Lu et al., 2011). They could interact with elevated CO_2 and modify the response of nitrification and denitrification to elevated CO_2 as suggested by the few individual studies that have investigated interactive effects among CO_2 and other global change factors and have reported significant interactions on nitrification and denitrification (Larsen et al., 2011; Zhang et al., 2017).

Here, we seek to understand the collective response of nitrification and denitrification rates and related functional genes to elevated CO_2 alone or combined with other global change factors. We conducted a meta-analysis based on 879 observations from

58 publications and 46 independent elevated CO_2 experiments in terrestrial ecosystems, including cropland, grassland and forest ecosystems. Our objectives were to (i) assess the responses of nitrification and denitrification rates and related functional genes to elevated CO_2 alone or combined with warming, increased precipitation, drought, and N addition, (ii) investigate the interactive effects of elevated CO_2 and warming, elevated CO_2 and increased precipitation or drought, and elevated CO_2 and N addition, (iii) analyze to what extent responses of nitrification and denitrification rates and related functional genes to elevated CO_2 are linked, and (iv) examine whether these responses to elevated CO_2 differ among ecosystem types and climate and soil characteristics.

2 | MATERIALS AND METHODS

2.1 | Data collection

We searched journal articles using the Web of Science Core Collection database and the search equation *((elevat* OR rising OR enrich*) NEAR/2 (CO2 OR "carbon dioxide")) AND (*nitrif* OR amoA OR AOA OR AOB OR nirK OR nirS OR nosZ* OR "functional genes")* in the title, abstract and keywords (349 results on July 18, 2022). Then, papers meeting the following criteria were selected: (i) research papers (not a review or a book chapter), (ii) including both control and CO_2 enrichment treatments with uninterrupted CO_2 application across the duration of the experiment, (iii) elevated CO_2 experiment conducted in a terrestrial ecosystem on a plant–soil system, (iv) at least one of the following variables was measured: gross or potential nitrification or denitrification rates, abundances of nitrifying and denitrifying functional genes (*amoA*-AOA, *amoA*-AOB, *nirK*, *nirS*, or *nosZ*), and (v) means, standard deviations or standard errors or confidence intervals, and sample sizes are available or extractable, either from tables, figures or the dataset when provided by the authors.

In each paper, we collected the following information: elevated CO_2 experiment location (GPS coordinates, or, if not reported, we estimated them using gps-coordinates.net; when different locations or when soils from different locations were studied in the same paper, they were considered as independent experiments), ecosystem type (cropland, grassland or forest), mean annual temperature and mean annual precipitation (if not reported, we estimated them using climate-data.org for experiments conducted in the field and with no irrigation), soil pH, magnitude of the CO_2 treatment, duration of the CO_2 treatment, other global change factors manipulated and crossed with the CO_2 treatment among elevated temperature, increased precipitation, drought, and N addition, magnitude of N addition in the elevated CO_2 experiment.

Data were extracted manually from tables or graphically from figures using WebPlotDigitizer 4.5 (Rohatgi, 2021), or were computed based on the dataset when provided by the authors for gross or potential nitrification or denitrification rates, abundances of nitrifying and denitrifying functional genes (*amoA*-AOA, *amoA*-AOB, *nirK*, *nirS*, or *nosZ*), N_2O emission rates and putative drivers of nitrifying and denitrifying microbial activities, including soil water content (gravimetric or volumetric soil water content or water-filled pore space), soil dissolved organic carbon (DOC) content, soil NH_4^+ and NO_3^- contents, gross N mineralization rates and gross N immobilization rates, soil pH, and plant biomass (aboveground, belowground, or total plant biomass). When several sampling dates were available in a single study, we extracted all data on nitrification and denitrification rates and related functional genes, and we extracted data on N_2O emission rates and putative drivers of nitrifying and denitrifying microbial activities for the measurement dates that were the closest to the dates of measurements of nitrification and denitrification rates and related functional genes. Negative values of N_2O emission rates and gross N transformation rates were excluded, as they do not enable to compute an effect size based on the natural log of the response ratio. When several sampling dates, sampling depths, or sampling modalities (e.g., rhizosphere versus bulk soil) were available in a single study, we extracted all data and considered them as multiple outcomes from the same elevated CO_2 experiment. Similarly, when several plant species or plant community composition, or different species management were manipulated in a single study, we considered them as multiple outcomes from the same elevated CO_2 experiment. When pollutants were manipulated in addition to the CO_2 treatment, we extracted the data from the treatment with no pollutant (e.g., cadmium (Jia et al., 2021), chlorpyrifos (Kollah et al., 2019) or tropospheric ozone (Kanerva et al., 2006)). Data were extracted for the control and the elevated CO_2 treatments (when several elevated levels of CO_2 were manipulated, data were extracted for each high CO_2 treatment, see Maxwell et al., 2022). If other global change factors than elevated CO_2 were manipulated among elevated temperature, increased precipitation, drought, and N addition, data for the control and the elevated CO_2 treatments were extracted for each level of the other manipulated global change factor.

Overall, a total of 879 observations (including 456 observations of nitrification and denitrification rates and abundances of nitrifying and denitrifying functional genes) taken from 58 papers (Table S1) and 46 independent elevated CO_2 experiments were reported. The global distribution of the elevated CO_2 experiments included in the meta-analysis is shown in Figure 1 (along with the data that were reported in each location) and Figure S1 (along with the ecosystem type studied at each location), and the distribution of mean annual temperature and mean annual precipitation of the experimental sites is shown in Figure S2.

2.2 | Data analysis

The natural log of the response ratio, defined as the effect size, was used to assess the responses of the variables studied to elevated CO_2 (Hedges et al., 1999).

The natural log of the response ratio ($\ln R$) and its associated variance (v) were calculated as:

$$\ln R = \ln \frac{\overline{X_t}}{\overline{X_c}} \quad (1)$$

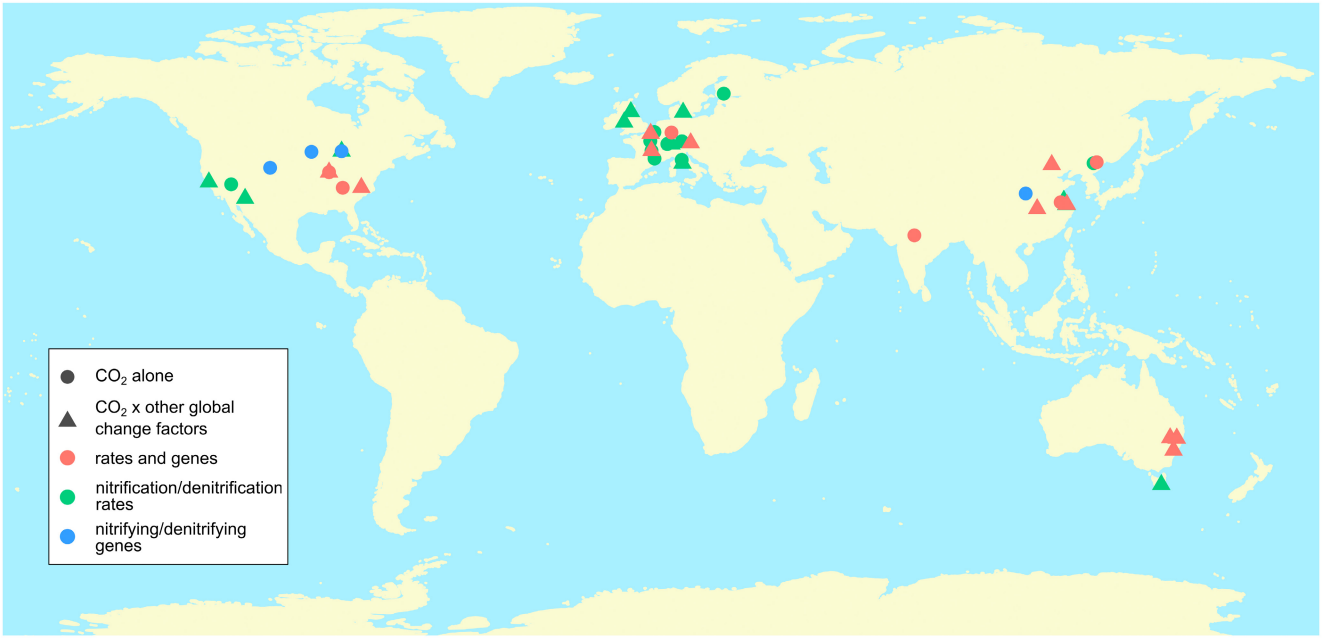

FIGURE 1 Global distribution of the elevated CO_2 experiments included in the meta-analysis. The shape shows if elevated CO_2 was manipulated alone (circle) or if it was combined with other global change factors among elevated temperature, increased precipitation, drought, and N addition (triangle). The color indicates the data that were reported in each location, either nitrification and/or denitrification rates and related functional genes (in red), nitrification and/or denitrification rates (in green), or nitrifying and/or denitrifying functional genes (in blue).

$$v = \frac{s_t^2}{n_t \overline{X_t^2}} + \frac{s_c^2}{n_c \overline{X_c^2}} \quad (2)$$

where $\overline{X_c}$ and $\overline{X_t}$ are the means of the control and elevated CO_2 treatments, s_c and s_t are the standard deviations of the control and elevated CO_2 treatments, and n_c and n_t are the number of replicates of the control and elevated CO_2 treatments.

Because we anticipate between-study heterogeneity in the effect sizes, we used a random-effects model to determine the total effect size with the restricted maximum likelihood (REML) estimator to compute the variance of the distribution of true effect sizes τ^2 (Viechtbauer, 2005). We further used Knapp–Hartung adjustments to calculate the confidence interval of the total effect size (Knapp & Hartung, 2003). For each study, the weighting factor (W_i) was calculated as the inverse of the sum of the pooled variance and heterogeneity for each treatment. When multiple observations were used from the same elevated CO_2 experiment, we adjusted the weights by the total number of observations per experiment (n_i) to get the final weights (W_i') (Bai et al., 2013):

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

$$W_i' = \frac{W_i}{n_i} \quad (4)$$

The total effect size ($\overline{\ln R}$) was estimated as the weighted average of all effect sizes:

$$\overline{\ln R} = \frac{\sum_i W_i' \ln R_i}{\sum_i W_i'} \quad (5)$$

where $\ln R_i$ is the ith individual effect size.

To facilitate the description of the results, the total effect size was then transformed back to the percentage change induced by the elevated CO_2 treatment as follows:

$$CO_2 \text{ effect}(\%) = \left(e^{\overline{\ln R}} - 1\right) \times 100\% \quad (6)$$

To further explore the two-way interactive effects of elevated CO_2 and other global change factors, among elevated temperature, increased precipitation, drought, and N addition, we used Hedge's d, an estimate based on standardized mean difference that was adapted for the assessment of interaction (Gurevitch et al., 2000). Hedge's d was computed for each two-way interaction and for each variable among nitrification and denitrification rates and abundances of related functional genes for which sufficient studies were available.

The main effects of the global change factors A and B (d_A and d_B) and their interaction ($d_{A\times B}$) were calculated as:

$$d_A = \frac{\left(\overline{X_A} + \overline{X_{A\times B}}\right) - \left(\overline{X_B} + \overline{X_C}\right)}{2s} J(m) \quad (7)$$

$$d_B = \frac{\left(\overline{X_B} + \overline{X_{A\times B}}\right) - \left(\overline{X_A} + \overline{X_C}\right)}{2s} J(m) \quad (8)$$

$$d_{A\times B} = \frac{\left(\overline{X_{A\times B}} - \overline{X_A}\right) - \left(\overline{X_B} - \overline{X_C}\right)}{2s} J(m) \quad (9)$$

where $\overline{X_A}, \overline{X_B}, \overline{X_{A\times B}}$, and $\overline{X_C}$ are the means in the groups A, B, and their combination *A* x *B*, and in the control group; *s* is the pooled standard deviation and *J*(*m*) is the correction term for small sample bias (Hedges & Olkin, 1985). *s* and *J*(*m*) were calculated as:

$$s = \sqrt{\frac{(n_C-1)\sigma_C^2 + (n_A-1)\sigma_A^2 + (n_B-1)\sigma_B^2 + (n_{A\times B}-1)\sigma_{A\times B}^2}{n_C + n_A + n_B + n_{A\times B} - 4}} \quad (10)$$

$$J(m) = 1 - \frac{3}{4(n_C + n_A + n_B + n_{A\times B} - 4) - 1} \quad (11)$$

where n_C, n_A, n_B, and $n_{A\times B}$ are the sample sizes in the control group, groups A and B, and their combination *A* x *B*, and $\sigma_C, \sigma_A, \sigma_B$, and $\sigma_{A\times B}$ are the standard deviations in the control group, groups A and B, and their combination *A* x *B*. The variance (v_{d_i}) of Hedge's *d* for a given treatment *i* (A, B or *A* x *B*) was calculated as:

$$v_{d_i} = \frac{\frac{1}{n_C} + \frac{1}{n_A} + \frac{1}{n_B} + \frac{1}{n_{A\times B}} + \frac{d_i^2}{2(n_C + n_A + n_B + n_{A\times B})}}{4} \quad (12)$$

The total effect size of each global change factor A and B and their interaction *A* x *B* were assessed using a random-effects model as described above with a REML estimator and Knapp–Hartung adjustments. We classified the interaction between A and B into additive (if 95% confidence interval overlapped zero), synergistic and antagonistic interactions. When the individual effect sizes of global change factors A and B were positive, an effect size of the interaction greater than zero was classified as synergistic and an effect size of the interaction lower than zero as antagonistic. When the individual effect sizes were negative, an effect size of the interaction greater than zero was classified as antagonistic and an effect size of the interaction lower than zero as synergistic. When the individual effect sizes were in opposite directions, the interaction was classified as synergistic when it had the same sign as the expected combined effect under an additive regime, and as antagonistic when the sign of the interaction was opposite to the expected combined effect under an additive regime (Crain et al., 2008; Li, Ma, et al., 2022; Wang et al., 2021).

Relationships between effect sizes of elevated CO_2 on the variables studied were investigated using correlations and linear regressions with adjusted *R*-squared. Meta-regressions were conducted with either a categorical variable (ecosystem type) or continuous variables (mean annual temperature, mean annual precipitation, soil pH, duration of the CO_2 treatment, magnitude of the CO_2 treatment, or magnitude of N addition in the elevated CO_2 experiment) used as moderators for the random-effects model (mixed-effects meta-regression model). For each categorical or continuous variable, statistical results were reported as between-group heterogeneity of effect sizes (QM), residual error (QE), and *p*-value from mixed-effects meta-regression model.

All statistical analyses were conducted using R 4.0.3 software (R Core Team, 2022) and the *meta* (Balduzzi et al., 2019) and *metafor* (Viechtbauer, 2010) packages.

3 | RESULTS

3.1 | Effects of elevated CO_2 across all terrestrial ecosystems and as a function of ecosystem type

Overall, elevated CO_2 significantly increased the abundances of denitrifying functional genes *nirK* (+25%, $p = .008$) and *nirS* (+27%, $p < .001$) and induced a marginally significant increase in the abundances of bacterial *amoA* functional gene (+16%, $p = .08$) and of denitrifying functional gene *nosZ* (+16%, $p = .07$) (Figure 2a). Increases in the abundances of bacterial *amoA* functional gene and denitrifying functional genes *nirK*, *nirS*, and *nosZ* in response to elevated CO_2 were more pronounced and all significant in cropland ecosystems (*amoA-AOB*: +62%, $p = .003$; *nirK*: +36%, $p = .006$; *nirS*: +30%, $p = .002$ and *nosZ*: +32%, $p = .006$) (Figure 2b), while abundances of bacterial *amoA* functional gene and denitrifying functional genes *nirK*, *nirS*, and *nosZ* were not significantly altered by elevated CO_2 in grassland and forest ecosystems (Figure 2b). In contrast to other functional genes, the abundance of archaeal *amoA* functional gene was unresponsive to elevated CO_2 (Figure 2a,b). In addition to the increases in the abundances of bacterial *amoA*, *nirK*, *nirS*, and *nosZ* functional genes, elevated CO_2 also significantly increased potential nitrification in cropland ecosystems (+28%, $p = .03$) and induced a marginally significant increase in gross nitrification in forest ecosystems (+23%, $p = .07$) (Figure 2b). Elevated CO_2 had no significant effect on potential denitrification rates across all terrestrial ecosystems (Figure 2a,b).

In the studies selected in the present meta-analysis, elevated CO_2 induced a marginally significant increase in soil N_2O emissions (+26%, $p = .06$; Figure 3). In addition, elevated CO_2 significantly increased plant biomass (+23%, $p = .008$) and soil moisture (+5%, $p = .03$) and significantly decreased soil NO_3^- content (−18%, $p = .005$) (Figure 3), while it had no significant overall effect on soil DOC content, gross N mineralization and N immobilization rates, soil NH_4^+ content, and soil pH (Figure 3).

3.2 | Effects of elevated CO_2 under elevated levels of other global change factors

Looking at the effects of elevated CO_2 under all possible combinations of elevated levels of other global change factors among elevated temperature, increased precipitation, drought, and N addition revealed that elevated CO_2 induced significant responses in potential denitrification rates under certain global change combinations: elevated CO_2 increased potential denitrification under N addition and higher precipitation (+116%, $p = .04$) and tended to decrease potential denitrification under elevated temperature (−15%, $p = .09$) (Figure 4a). Responses of gross nitrification rates, potential nitrification rates and abundances of nitrifying and denitrifying functional genes to elevated CO_2 were overall similar under all possible combinations of elevated levels of other global change factors (Figure 4a–c), except for the abundance of *nosZ* that exhibited a significant and

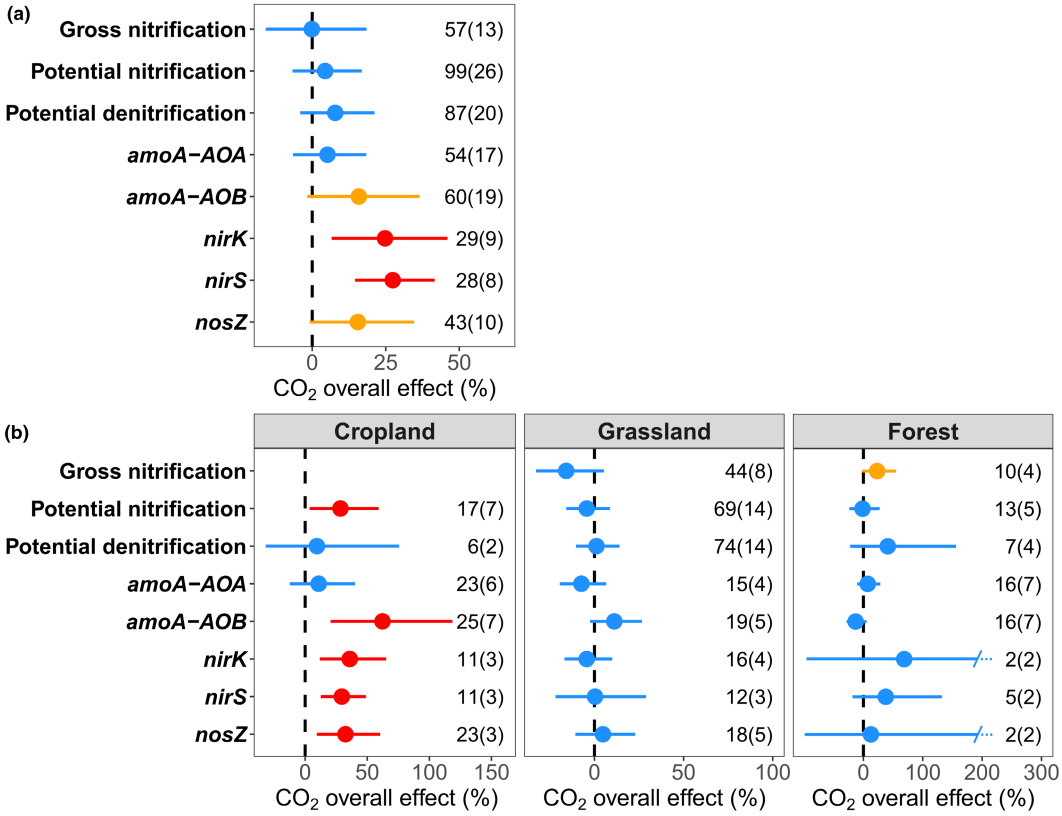

FIGURE 2 Effects of elevated CO_2 on nitrification and denitrification rates and nitrifying and denitrifying functional genes across all terrestrial ecosystems (a) and as a function of ecosystem type (cropland, grassland or forest) (b). Mean effect sizes were expressed as percentage changes resulting from elevated CO_2. Error bars indicate 95% confidence intervals. The blue slash with dots indicates a confidence interval going outside of the box. The dashed line was drawn at the mean effect equal to zero. Effects were considered significant if the 95% confidence interval did not overlap zero. Significant effects ($p < .05$) are indicated in red, marginally significant effects ($p < .1$) in orange, and non-significant effects ($p > .1$) in blue. The number of observations for each variable is shown next to the point, and the number of independent experiments is indicated in parentheses.

larger increase in response to elevated CO_2 when associated with N addition and elevated temperature (CO_2+N+T: +62%, $p = .03$; Figure 4c).

3.3 | Relationships between responses to elevated CO_2 of nitrification and denitrification rates and related functional genes

The effect size of elevated CO_2 on potential nitrification was significantly and positively correlated with the effect sizes of elevated CO_2 on the abundance of archaeal *amoA* functional gene ($p = .02$, $R^2 = .15$) and of bacterial *amoA* functional gene ($p = .02$, $R^2 = .13$) (Figure S3; Table S2), although with low *R*-squared of the corresponding linear regressions. The responses of most nitrifying and denitrifying functional genes to elevated CO_2 were significantly and positively correlated. In particular, the effect size of elevated CO_2 on bacterial *amoA* functional gene significantly increased with the effect size of elevated CO_2 on *nirK* ($p = .01$, $R^2 = .24$), *nirS* ($p = .02$, $R^2 = .23$) and *nosZ* ($p < .001$, $R^2 = .54$) functional genes (Figure S3; Table S2); the effect size of elevated CO_2 on *nirK* significantly increased with the effect size of elevated CO_2 on *nirS* ($p < .001$, $R^2 = .48$) and *nosZ* ($p < .001$, $R^2 = .55$) (Figure S3; Table S2) and the effect size of elevated CO_2 on *nirS* also significantly increased with the effect size of elevated CO_2 on *nosZ* ($p = .01$, $R^2 = .23$) (Figure S3; Table S2). In contrast, the relationships between effect sizes of elevated CO_2 on nitrification and denitrification rates and related functional genes and effect sizes of elevated CO_2 on putative drivers of nitrifying and denitrifying microbial activities were mostly not significant (Tables S3 and S4).

3.4 | Interactive effects of elevated CO_2 with other global change factors

Overall, there were few available studies on the combined effects of elevated CO_2 and other global change factors among elevated temperature, increased precipitation, drought, and N addition to investigate interactive effects on nitrification and denitrification and related functional genes (Figure 5; Figure S4). Among the 14 interactions tested between elevated CO_2 and elevated temperature, elevated CO_2 and increased precipitation,

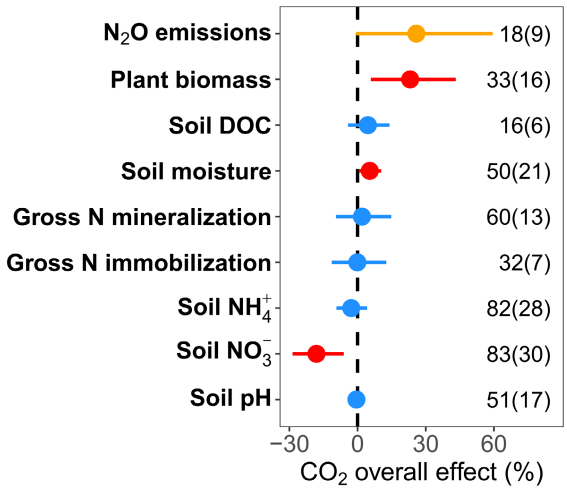

FIGURE 3 Effects of elevated CO_2 on soil N_2O emissions and putative drivers of nitrifying and denitrifying microbial activities. Mean effect sizes were expressed as percentage changes resulting from elevated CO_2. Error bars indicate 95% confidence intervals. The dashed line was drawn at the mean effect equal to zero. Effects were considered significant if the 95% confidence interval did not overlap zero. Significant effects ($p < .05$) are indicated in red, marginally significant effects ($p < .1$) in orange, and non-significant effects ($p > .1$) in blue. The number of observations for each variable is shown next to the point, and the number of independent experiments is indicated in parentheses.

and elevated CO_2 and N addition, four interactions were marginally significant: an antagonistic interaction between elevated CO_2 and elevated temperature on potential nitrification (Hedge's $d = -.23$, $p = .07$; Figure 5a), a synergistic interaction between elevated CO_2 and increased precipitation on potential denitrification (Hedge's $d = .47$, $p = .052$; Figure 5b), a synergistic interaction between elevated CO_2 and N addition on the abundance of archaeal *amoA* functional gene (Hedge's $d = -.12$, $p = .052$; Figure 5c), and a synergistic interaction between elevated CO_2 and elevated temperature on the abundance of *nosZ* functional gene (Hedge's $d = .23$, $p = .08$; Figure 5d). Additive effects of elevated CO_2 and elevated temperature, elevated CO_2 and increased precipitation, and elevated CO_2 and N addition were found for 10 out of 14 interactions tested (i.e., the effect size of the interaction was not significantly different from zero for 10 out of 14 interactions tested; Figure S4).

3.5 | Relationships between responses to elevated CO_2 of nitrification and denitrification rates and related functional genes and main characteristics of ecosystems and CO_2 experiments

Mean annual temperature and mean annual precipitation had significant and positive effects on the effect size of elevated CO_2 on potential nitrification ($p = .009$ and $p < .001$; Figure 6a,b; Table S5), on the abundance of bacterial *amoA* functional gene ($p = .01$ and $p < .001$; Figure 6d,e; Table S6) and on the abundance of *nirS* functional gene ($p = .004$ and $p < .001$; Figure 6g,h; Table S6). Soil pH had also a significant and positive effect on the effect size of elevated CO_2 on potential nitrification ($p = .002$; Figure 6c; Table S5) and on the abundance of bacterial *amoA* functional gene ($p = .02$; Figure 6f; Table S6). The effect size of elevated CO_2 on the abundance of bacterial *amoA* functional gene further significantly increased with the magnitude of N addition in the elevated CO_2 experiments ($p = .002$; Figure 6i; Table S6).

3.6 | Effects of elevated CO_2 as a function of climate and soil characteristics

In the elevated CO_2 experiments where the mean annual temperature was lower than 10°C (the mid-range of mean annual temperature in the temperate zone, Woodward et al., 2004), elevated CO_2 had no significant effect on nitrification and denitrification rates and related functional genes, while in the study sites where the mean annual temperature was higher than 10°C, elevated CO_2 significantly increased the abundances of bacterial *amoA* (+39%, $p = .004$), *nirK* (+43%, $p = .02$) and *nirS* (+48%, $p = .001$) functional genes (Figure S5) and induced a marginally significant increase in potential denitrification (+19%, $p = .08$; Figure S5). In the elevated CO_2 experiments where the mean annual precipitation was lower than 800 mm (the average mean annual precipitation in the temperate zone, Woodward et al., 2004), elevated CO_2 had no significant effect on nitrification and denitrification rates and related functional genes, while in the study sites where the mean annual precipitation was higher than 800 mm, elevated CO_2 significantly increased the abundances of bacterial *amoA* (+55%, $p = .0004$) and *nirS* (+39%, $p = .002$) functional genes (Figure S6) and induced a marginally significant increase in potential nitrification (+20%, $p = .06$; Figure S6).

The effect of elevated CO_2 on potential nitrification and abundances of some nitrifying and denitrifying functional genes varied across soil pH (Figure S7): elevated CO_2 significantly increased potential nitrification in alkaline soils (+31%, $p = .008$), while it had no significant effect in neutral soils and induced a marginally significant decrease in potential nitrification in acidic soils (−15%, $p = .09$) (Figure S7); elevated CO_2 significantly increased the abundance of bacterial *amoA* functional gene in neutral (+41%, $p = .0009$) and alkaline soils (+42%, $p = .02$), while it had no significant effect on bacterial *amoA* in acidic soils (Figure S7); elevated CO_2 significantly increased the abundance of *nosZ* functional gene in neutral soils (+33%, $p = .01$), while it had no significant effect on *nosZ* in acidic soils (Figure S7).

4 | DISCUSSION

Overall, and as summarized in Figure 7, our meta-analysis shows that elevated CO_2 induced increases in both nitrification and denitrification rates and abundances of most nitrifying and denitrifying functional genes and

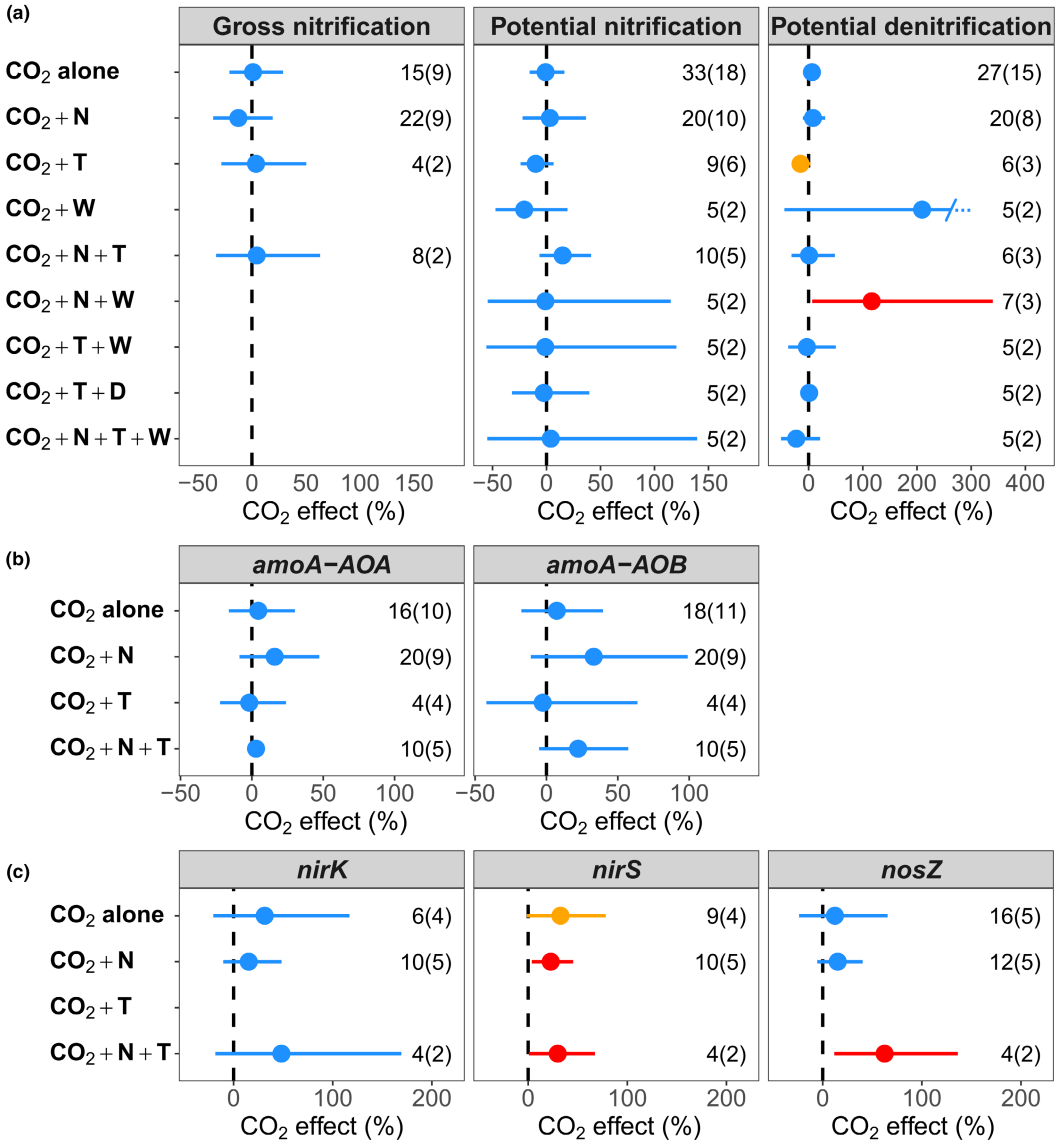

FIGURE 4 Effects of elevated CO_2 alone or under elevated levels of other global change factors among N addition (+N), elevated temperature (+T), increased precipitation (+W), or drought (+D) on nitrification and denitrification rates (a), nitrifying functional genes (b), and denitrifying functional genes (c). The treatments are indicated as defined by the authors of the individual studies, except for N addition, where all studies with N addition greater than 30 kg N ha^{-1} $year^{-1}$ were included. Mean effect sizes were expressed as percentage changes resulting from elevated CO_2. Error bars indicate 95% confidence intervals. The blue slash with dots indicates a confidence interval going outside of the box. The dashed line was drawn at the mean effect equal to zero. Effects were considered significant if the 95% confidence interval did not overlap zero. Significant effects ($p < .05$) are indicated in red, marginally significant effects ($p < .1$) in orange, and non-significant effects ($p > .1$) in blue. The number of observations for each variable is shown next to the point, and the number of independent experiments is indicated in parentheses.

that some of these positive responses to elevated CO_2 were more pronounced or occurred only in cropland ecosystems, or under particular global change scenarios, or particular climate and soil characteristics.

4.1 | Effects of elevated CO_2 on nitrification rates and nitrifying functional genes

Elevated CO_2 induced significant increases in potential nitrification rates and in the abundance of bacterial *amoA* functional gene in cropland ecosystems (the effect was marginally significant across all terrestrial ecosystems), while the abundance of archaeal *amoA* functional gene was unresponsive to elevated CO_2. Previous meta-analyses had reported no response or decreases in nitrification rates under elevated CO_2 (Barnard et al., 2005; Liang et al., 2016), in particular in long-term CO_2 experiments and in ecosystems where no N was added (Liang et al., 2016), and negative responses of nitrification have been attributed to decreased NH_4^+ availability for nitrifiers under elevated CO_2. The increase in the abundance of bacterial *amoA* functional gene in cropland ecosystems occurred along with no significant change in the abundance of archaeal *amoA* functional gene, which is in agreement with a previous meta-analysis assessing the effects of

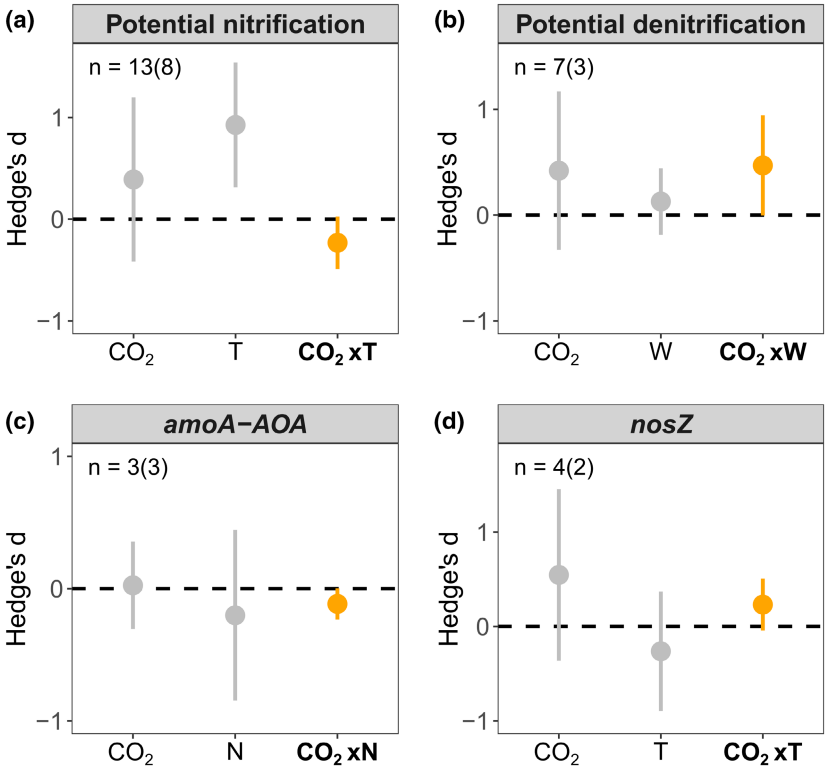

FIGURE 5 Individual effects and interactive effects of elevated CO_2 and other global change factors among N addition (N), elevated temperature (T), and increased precipitation (W) on potential nitrification (a), potential denitrification (b), archaeal *amoA* functional gene (c) and *nosZ* functional gene (d). Values are Hedge's *d* of elevated CO_2, N addition, elevated temperature, increased precipitation and their combinations ($CO_2 \times N$, $CO_2 \times T$, $CO_2 \times W$). Error bars indicate 95% confidence intervals. The dashed line was drawn at Hedge's d equal to zero. Interactive effects were considered significant if the 95% confidence interval did not overlap zero. Significant interactive effects ($p < .05$) are indicated in red, marginally significant interactive effects ($p < .1$) in orange, and non-significant interactive effects ($p > .1$) in blue. The number of observations is specified in each panel, and the number of independent experiments assessing the investigated two-way interaction is indicated in parentheses.

elevated CO_2 on nitrifying functional genes in agricultural soils (Du et al., 2022) and supports the idea of a niche differentiation between AOA and AOB microbial communities in soils (Prosser & Nicol, 2012). A possible explanation for increases in potential nitrification rates and the abundance of bacterial *amoA* functional gene in response to elevated CO_2 in cropland ecosystems and for the marginally significant increase in gross nitrification rates in forest ecosystems may be related to increases in soil moisture resulting from decreased stomatal conductance at high CO_2, an effect that has been widely documented (van Groenigen et al., 2011) and is observed in our meta-analysis (Figure 7). Indeed, a relatively moderate elevation of soil moisture can increase nitrification rates and abundance of the nitrifying microbial communities by reducing water stress (Horz et al., 2004).

4.2 | Effects of elevated CO_2 on denitrification rates and denitrifying functional genes

Elevated CO_2 induced significant or marginally significant changes in potential denitrification rates but only under certain global change scenarios: elevated CO_2 significantly increased potential denitrification when combined with N addition and higher precipitation and tended to decrease potential denitrification when combined with elevated temperature. In addition, elevated CO_2 significantly increased the abundances of *nirK* and *nirS* (increases were more pronounced in cropland ecosystems). Elevated CO_2 also significantly increased the abundance of *nosZ* in cropland ecosystems (the effect was marginally significant across all terrestrial ecosystems), and the increase in the abundance of *nosZ* in response to elevated CO_2 was remarkably larger when elevated CO_2 was combined with N addition and elevated temperature. Previous meta-analyses assessing the effects of elevated CO_2 on denitrification rates have reported both decreases in denitrification rates, although with a large variability among studies (Barnard et al., 2005), and increases in denitrification rates under elevated CO_2 in ecosystems where N was added (Liang et al., 2016). Increases in the abundances of *nirK* and *nirS* functional genes were previously observed in meta-analyses assessing the effects of elevated CO_2 on denitrifying functional genes in agricultural soils (Du et al., 2022) and across all terrestrial ecosystems for the abundance of *nirK* (Li, Ma, et al., 2022). In contrast, increases in the abundance of *nosZ* in response to elevated CO_2 in cropland ecosystems, or when elevated

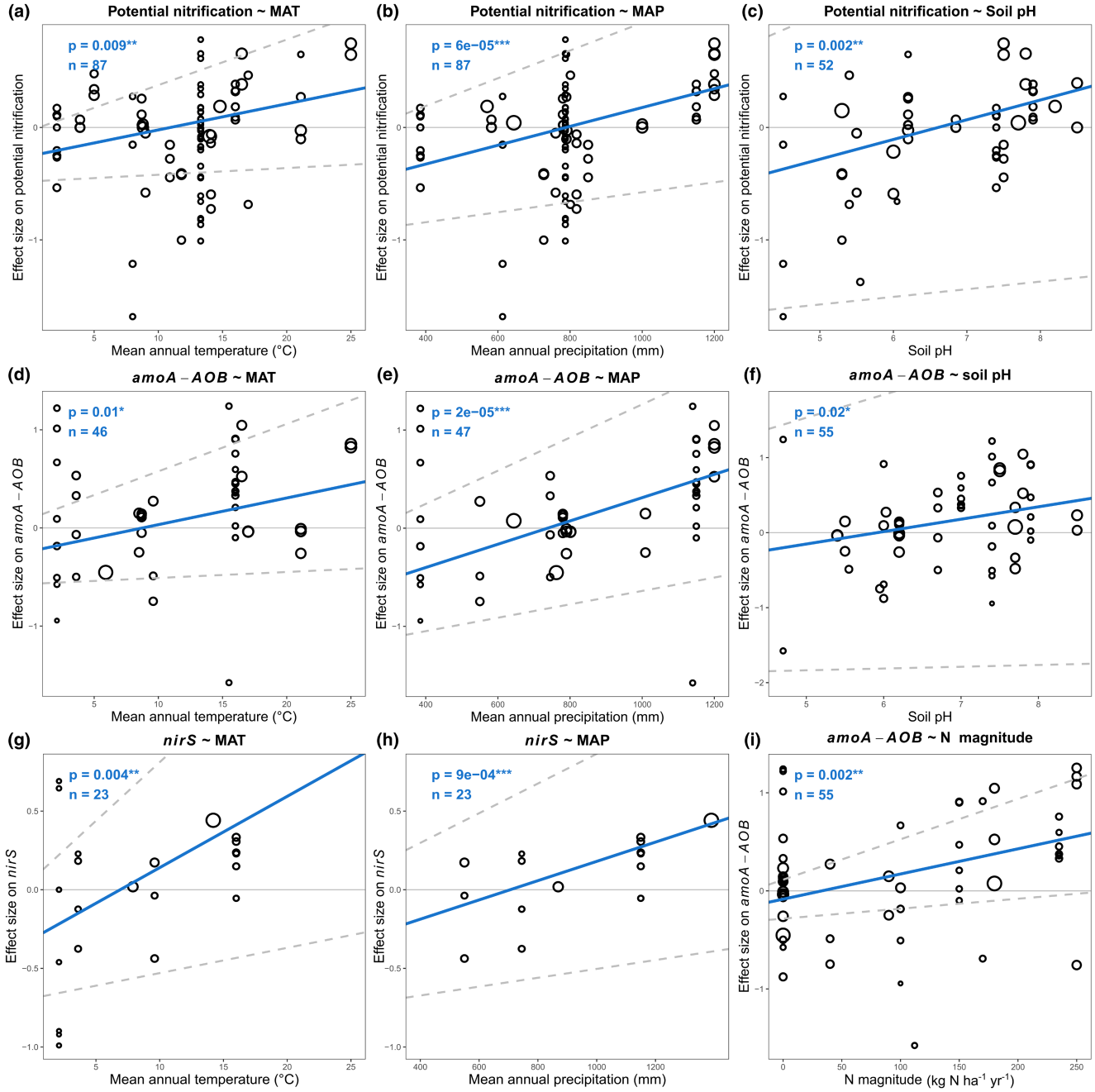

FIGURE 6 Relationships between the effect sizes of elevated CO_2 on potential nitrification rate or abundances of bacterial *amoA* and *nirS* functional genes and mean annual temperature MAT (a, d, g), mean annual precipitation MAP (b, e, h), soil pH (c, f), and magnitude of N addition (i) at the experimental site. In each panel, the blue line shows the regression line given by the mixed-effects meta-regression model, and the dotted lines represent the 95% confidence interval. The bubble size is proportional to the weight of the studies in the analysis (with larger points for studies with more weight). The *p*-value from the mixed-effects meta-regression model is indicated with stars encoding significance levels (***$p < .001$; **$p < .01$; *$p < .05$, see Tables S5 and S6), and the number of effect sizes included in the meta-regression is specified.

CO_2 was combined with N addition and elevated temperature, were not previously reported. A possible explanation for increases in potential denitrification rates with elevated CO_2, N addition and increased precipitation is related to the relaxation of multiple limiting factors of denitrifying microbial communities under the combined effects of these global change factors. First, increased soil moisture (Figure 7) and associated decreased soil oxygen content under elevated CO_2 should favor anaerobic denitrifying microbial communities, an effect that was likely more pronounced when elevated CO_2 was associated with increased precipitation (Brown et al., 2012). Second, increased plant biomass in response to elevated CO_2, a response that is widely documented (Ainsworth & Long, 2005) and observed in our meta-analysis (Figure 7), should result in higher soil labile C availability through increased inputs of root exudates and litter to soils and favor heterotrophic denitrifying microbial communities (Baggs et al., 2003). Third, N addition

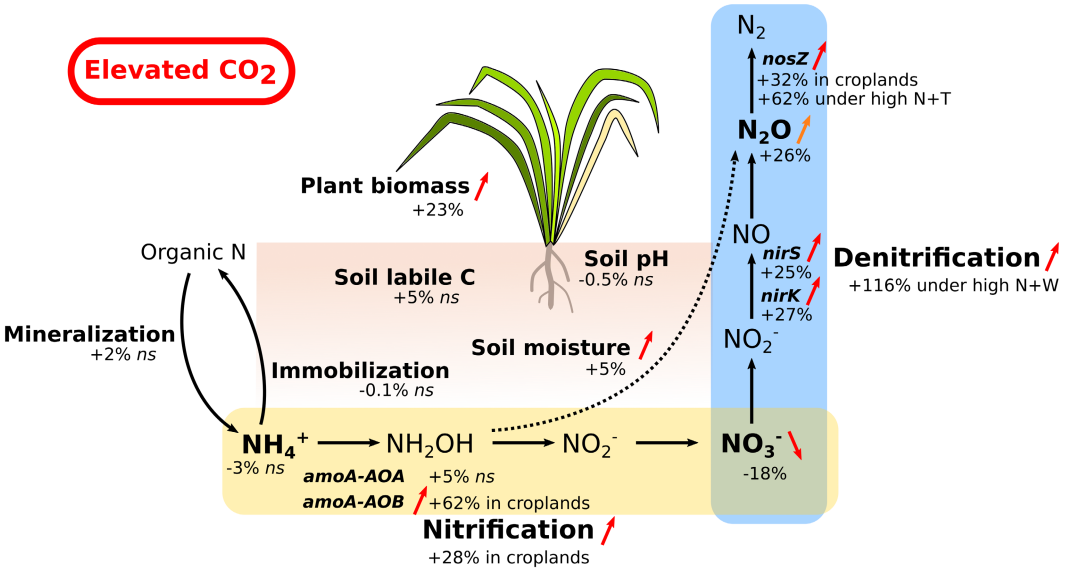

FIGURE 7 Diagram illustrating how elevated CO_2 altered nitrification and denitrification rates and abundances of related functional genes across terrestrial ecosystems. Responses to elevated CO_2 of putative drivers of nitrifying and denitrifying microbial activities investigated in the meta-analysis are also shown. Percentage changes resulting from elevated CO_2 are indicated. The arrows next to the variables indicate significant (in red) or marginally significant (in orange) effects of elevated CO_2 on the corresponding variables, ns indicates a non-significant effect. Effects occurring only in croplands or under certain global change scenarios are indicated (high N + W: N addition and increased precipitation; high N + T: N addition and elevated temperature).

should increase mineral N availability and relax N limitation of denitrification (Niboyet et al., 2010; Niboyet, Le Roux, et al., 2011). Similar mechanisms (increased soil moisture, decreased soil oxygen content, and increased soil labile C content) are also likely to be responsible for the observed increases in the abundances of *nirK*, *nirS* and *nosZ* denitrifying functional genes in response to elevated CO_2, especially in cropland ecosystems (Attard et al., 2011).

4.3 | Relationships between responses of nitrification and denitrification rates and related functional genes to elevated CO_2

Overall, the responses of bacterial *amoA*, *nirK*, *nirS*, and *nosZ* functional genes were well related as indicated by concurrent increases in the abundances of these functional genes with CO_2 enrichment and as confirmed by the significant and positive relationships between the effect sizes of elevated CO_2 on these different functional genes, which suggests that CO_2 enrichment induced positive effects on total soil bacteria, including the nitrifying and denitrifying microbial communities. Increases in the abundances of the nitrifying and denitrifying functional genes under elevated CO_2 mostly translated into increases in potential nitrification (in cropland ecosystems) and in potential denitrification (when CO_2 was combined with N addition and increased precipitation) and are consistent with the observed increase in soil N_2O emissions in our meta-analysis (+26%, Figure 7) as observed in previous meta-analyses on the effects of elevated CO_2 on soil N_2O emissions (Du et al., 2022; Liang et al., 2016; van Groenigen et al., 2011; Wang et al., 2021).

The response of potential nitrification to elevated CO_2 was consistent with the response of bacterial *amoA* functional gene, and we found significant and positive relationships between the effect size of elevated CO_2 on potential nitrification and on the abundances of bacterial and archaeal *amoA* functional genes, although the effect size of elevated CO_2 on the abundances of *amoA* functional genes only explained 13%–15% of the variance of the effect size of elevated CO_2 on potential nitrification. Such positive relationships between nitrification rates and abundances of nitrifying genes were not previously reported in the only meta-analysis on the impact of global change on soil N cycling that also assessed the responses of both nitrification and denitrification rates and abundances of related functional genes (Dai et al., 2020) and suggest that abundances of nitrifying functional genes may well reflect abundances of functionally active nitrifying enzymes in the soils under elevated CO_2. In contrast, the response of potential denitrification to elevated CO_2 shows some discrepancies with those of denitrifying functional genes, and we found no significant relationships between the effect sizes of elevated CO_2 on potential denitrification and on the abundances of *nirK*, *nirS* and *nosZ* functional genes, an apparent paradox that could be due to no expression of these functional genes. Under elevated CO_2, soil environmental conditions were likely favorable for denitrifying microbial communities, but the amount of functionally active denitrifying enzymes may have been hindered by low substrate availability, decreasing functional genes expression and thus potential denitrification. Consistent with this, soil NO_3^- content significantly decreased in response to elevated CO_2 (Figure 7), an effect that could be due to increased plant and microbial N demand associated with increased

plant and microbial biomass under elevated CO_2 and/or with increased leaching of NO_3^- resulting from increased soil moisture under CO_2 enrichment (Barnard et al., 2005).

4.4 | Interactive effects of elevated CO_2 and other global change factors on nitrification and denitrification rates and related functional genes

We investigated all possible two-way interactions between elevated CO_2 and other global change factors among elevated temperature, increased precipitation, drought, and N addition. To date, the limiting number of available studies of interactive effects constrained our capacity of detecting significant interactions between elevated CO_2 and other global change factors on nitrification and denitrification rates and related functional genes (Niboyet, Brown, et al., 2011; Niboyet, Le Roux, et al., 2011). However, we found four marginally significant interactive effects among elevated CO_2 and other global change factors ($CO_2 \times T$ on potential nitrification, $CO_2 \times W$ on potential denitrification, $CO_2 \times N$ on archaeal *amoA*, and $CO_2 \times T$ on *nosZ*). Among those, synergistic interactions were dominant, such as the interaction between elevated CO_2 and increased precipitation on potential denitrification, with a positive combined effect of elevated CO_2 and increased precipitation on potential denitrification that was larger than expected based on an additive assumption. A likely explanation is that elevated CO_2 and increased precipitation, by increasing soil moisture and soil labile C, induced optimal conditions for denitrification, resulting in amplifying effects of these global change factors when they were combined. To our knowledge, only two meta-analyses have statistically investigated interactive effects among global change factors on soil N cycling: Wang et al., 2021 assessed the interactive effect of elevated CO_2 and elevated temperature on soil N_2O emissions and reported a synergistic interaction, while Li, Ma, et al. (2022) assessed the interactive effects of warming and N addition, warming and increased precipitation, and warming and decreased precipitation on abundances of N cycling functional genes and reported a synergistic interaction of warming and N addition. Synergistic interactions on soil N cycling were thus dominant in our meta-analysis and in the literature, which clearly highlights the need for further investigations on interactive effects, as we could underestimate the effects of multiple global change factors on nitrification and denitrification and associated soil N_2O emissions.

4.5 | Effects of elevated CO_2 vary across ecosystem types and climate and soil characteristics

Increases in potential nitrification and the abundances of bacterial *amoA*, *nirK*, *nirS* and *nosZ* functional genes in response to elevated CO_2 occurred in cropland ecosystems and not in grassland and forest ecosystems, where no significant changes in nitrification and denitrification rates and abundances of related functional genes were found (except a marginally significant increase in gross nitrification in forest ecosystems). Agricultural soils often receive substantial amounts of N fertilizer (up to 265 kg N ha^{-1} $year^{-1}$ in our study), so that nitrifying and denitrifying microbial communities were likely not, or at least less, constrained by mineral N availability in these soils compared with grassland and forest soils, and could thus benefit from the relaxation of other limiting factors of nitrification and denitrification induced by elevated CO_2 (e.g., increased soil moisture and soil labile C content). In agreement with this and with the preference of AOB for soils with high N content (Prosser & Nicol, 2012), increases in the abundance of bacterial *amoA* functional gene with elevated CO_2 increased with the amount of added N in the elevated CO_2 experiments.

The effect of elevated CO_2 on nitrification varied across soil pH. Increases in potential nitrification occurred in alkaline soils, and increases in the abundance of bacterial *amoA* functional gene occurred in neutral and alkaline soils. Furthermore, the magnitude of increases in potential nitrification and the abundance of bacterial *amoA* functional gene in response to elevated CO_2 significantly increased with soil pH. At a global scale, soil nitrification rate has been shown to increase with soil pH, with higher nitrification rates in alkaline soils, as lower soil pH may dampen the soil microbial activity of nitrification, reduce richness and diversity of ammonia-oxidizers, and provide less substrate for soil nitrification (Li, Zeng, et al., 2020). Alkaline soils are known to be common in arid climates (Slessarev et al., 2016), which may thus be particularly sensitive to rising atmospheric CO_2 for the nitrification microbial process.

The effect of elevated CO_2 on nitrification and denitrification and related functional genes also varied across climate characteristics. Increases in the abundances of nitrifying and denitrifying functional genes in response to elevated CO_2 occurred in ecosystems with a mean annual temperature higher than 10°C (for bacterial *amoA*, *nirK* and *nirS*, along with a marginally significant increase in potential denitrification) and in ecosystems with a mean annual precipitation higher than 800 mm (for bacterial *amoA* and *nirS*, along with a marginally significant increase in potential nitrification), while nitrification and denitrification rates and abundances of related functional genes remained unaffected by elevated CO_2 in ecosystems with a mean annual temperature lower than 10°C or a mean annual precipitation lower than 800 mm. Furthermore, the magnitude of increases in potential nitrification and in the abundances of bacterial *amoA* and *nirS* functional genes in response to elevated CO_2 significantly increased with mean annual temperature and mean annual precipitation. Global syntheses on the controlling factors of soil nitrification and denitrification have shown that soil nitrification rate increased with mean annual temperature (Li, Zeng, et al., 2020) and that soil denitrification rate increased with mean annual temperature and mean annual precipitation (Li, Tang, et al., 2022). Taken together, these results suggest that the highest nitrification and denitrification rates occur in ecosystems with the highest mean annual temperature and mean annual precipitation, which are also ecosystems where the largest increases in nitrification and denitrification rates and abundances of related functional genes in response to elevated CO_2 are expected. The global distribution of

elevated CO_2 experiments is clearly biased, with experiments mostly concentrated on the temperate zone (Figure 1, Figure S1), with a particular range of mean annual temperature and mean annual precipitation (Figure S2). Our meta-analysis clearly highlights the need for more global change experiments in biomes with higher mean annual temperature and mean annual precipitation, where elevated CO_2 might elicit the largest increases in nitrification and denitrification rates and associated N_2O emissions (e.g., in tropical rainforest, Woodward et al., 2004).

5 | CONCLUSIONS

Our meta-analysis shows that elevated CO_2 can cause increases in nitrification and denitrification rates and abundances of bacterial *amoA*, *nirK*, *nirS* and *nosZ* functional genes, resulting in increased soil N_2O emissions. We found that positive responses to elevated CO_2 were clearly most pronounced in cropland ecosystems and that responses to elevated CO_2 vary across soil pH and climate characteristics. Furthermore, some positive effects of elevated CO_2 occurred under certain global change scenarios (e.g., when CO_2 was combined with N addition and higher precipitation for potential denitrification), and our meta-analysis suggests potential interactions among CO_2 and other global change factors on nitrification and denitrification, resulting in effects that could be greater than those predicted based on single-factors experiments (i.e., synergistic interactions), which underlines the importance of assessing interactive effects among global change factors if we wish to be able to accurately predict the response of soil N cycling to global change. Our meta-analysis further shows that the magnitude of increases in potential nitrification and abundances of bacterial *amoA* and *nirS* functional genes under elevated CO_2 increased with mean annual temperature and mean annual precipitation and stresses the need for studies in biomes outside the temperate zone, in particular in the tropical zone, where the highest increases in nitrification and denitrification in response to elevated CO_2 might occur. Also, as the mean annual temperature is expected to increase globally and as the mean annual precipitation is expected to increase in large regions of the world (IPCC, 2021), the positive effects of elevated CO_2 on nitrification, denitrification, and associated N_2O emissions could be reinforced with ongoing climate change, thereby accelerating positive feedback on climate change.

ACKNOWLEDGMENTS

This study was supported by Sorbonne Université, AgroParisTech and the Ecole Normale Supérieure of Lyon.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Zenodo at https://zenodo.org/record/7457227#.Y6BEjHbMJPY.

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SUPPORTING INFORMATION

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How to cite this article: Gineyts, R., & Niboyet, A. (2023). Nitrification, denitrification, and related functional genes under elevated CO_2: A meta-analysis in terrestrial ecosystems. *Global Change Biology*, *29*, 1839–1853. https://doi.org/10.1111/gcb.16568