Trade-offs on carbon and nitrogen availability lead to only a minor effect of elevated CO_2 on potential denitrification in soil

Can Liu[a], Roland Bol[b, c], Xiaotang Ju[d], Jing Tian[e, f], Di Wu[a, *]

[a]Beijing Key Laboratory of Biodiversity and Organic Farming, College of Resources and Environmental Sciences, China Agricultural University, Beijing, China

[b]Institute of Bio- and Geosciences, Agrosphere (IBG-3), Forschungszentrum Jülich GmbH, Jülich, Germany

[c]School of Natural Sciences, Environment Centre Wales, Bangor University, Bangor, United Kingdom

[d]College of Tropical Crops, Hainan University, Haikou, China

[e]National Academy of Agriculture Green Development, China Agricultural University, Beijing, China

[f]Key Laboratory of Plant-Soil Interactions, Ministry of Education, China Agricultural University, Beijing, China

***Corresponding author:** Di Wu. Telephone: 17801580063. E-mail: d.wu@cau.edu.cn

Abstract

The global surplus of reactive nitrogen (N_r) and elevated atmospheric CO_2 are both major threats to current and future ecosystem integrity. Denitrification is the primary sink for N_r by the conversion to inert N_2 and is also the predominant source of N_2O emission. Yet, it is unclear how and to what extent soil denitrification would respond to elevated CO_2 (eCO_2), which is a major part of the uncertainties on the estimation of terrestrial nitrogen (N) cycle budget under the influence of climate changes. Herein, we provided quantitative results of a global meta-analysis with 127 observations that included data on soil potential denitrification rates (PDR) and denitrifying gene abundance, and 305 observations that included data on N_2O emission and soil available N content under eCO_2. Averaged across all studies, we found that globally eCO_2 had an overall minor effect on soil PDR, but showed a large variation with different edaphic and environmental factors. The response was primarily explained by soil organic carbon (SOC) and mean annual precipitation (MAP), i.e., the higher SOC content or MAP, the lower response of soil PDR to eCO_2. Further, eCO_2 significantly decreased soil NO_3^- content (-15.3%) and denitrifying bacteria (-31.3%), but increased *nir*S (14.7%), *nap*A (24.4%) genes and N_2O emission (34.1%). We speculate that eCO_2 has both positive and negative effects on soil potential denitrification, i.e., a higher soil C input induced by eCO_2 supplies more energy for denitrifiers and thus increases denitrification in N-rich ecosystems, while unbalanced C:N input decreases soil NO_3^- content, causing substrate deficiency in N-limiting ecosystems under eCO_2. Whilst the response of soil PDR to eCO_2 is governed by trade-offs on soil C and N availability, this may serve as a buffer on soil available N content in terrestrial ecosystems under eCO_2. Collectively, based on our findings, we built a comprehensive conceptual model defining the key regulators, which has significant implications for expanding the understanding and predicting the responses of the terrestrial N cycle to eCO_2 in future scenario evaluation.

Keywords

elevated carbon dioxide; soil potential denitrification; nitrogen cycle; substrate availability; trade-off

1 Introduction

The amount of reactive nitrogen (N_r) applied to land has increased by 160% over the last two hundred years, leading to an unprecedented accumulation of N_r in terrestrial ecosystems (Butterbach-Bahl and Dannenmann, 2011; Scheer et al., 2020). Soil denitrification is the most important biological process to remove the excessive N_r in the terrestrial biosphere (Butterbach-Bahl and Dannenmann, 2011), which is also one of the main sources of emitted nitrous oxide (N_2O) from the soil, a potent greenhouse gas and ozone-depleting substance (IPCC, 2007; Ravishankara et al., 2009). Meanwhile, the concentration of atmospheric carbon dioxide (CO_2) has risen to ~50% above its pre-industrial level (from ~280ppm to ~420ppm) and is projected to continue rising by the end of this century even under the most ambitious mitigation scenario (IPCC, 2014, 2021). A major part of the uncertainties on the estimation of terrestrial N cycle budget under the influence of climate changes is related to how and to what extent soil denitrification would respond to elevated CO_2 (eCO_2), and whether the response would create a positive feedback loop on the greenhouse effect in the future eCO_2 scenario. However, the response of soil denitrification to CO_2 enrichment at the global scale remains unclear, with positive (Robinson and Conroy, 1998; Carnol et al., 2002; Baggs et al., 2003), negative (Tscherko et al., 2001; Billings et al., 2002) or non-significant responses reported (Larsen et al., 2011; Niboyet et al., 2011; Zhong et al., 2015). A global synthesis is needed to understand how denitrification would response to eCO_2 in various environments.

Plant-soil interactions under eCO_2 have complex impacts on soil denitrification process. Soil N availability, labile C availability, and soil oxygen status, the three key parameters that regulate the denitrification process, are regulated by plant-soil interactions and are all significantly affected by eCO_2 as well (Peralta and Wander, 2008; Terrer et al., 2018). Due to stimulated photosynthesis under eCO_2, enhanced plant biomass increases plant demand for N_r (Drigo et al., 2008), which decreases soil N availability and eventually constrains their own growth in turn, known as the Progressive Nitrogen Limitation (PNL) concept (Ainsworth and Long, 2005, 2021; Norby and Zak, 2011). In theory, the PNL would slow down soil N cycle processes such as nitrification and denitrification due to the lowering soil N availability, which was often proposed as the mechanism explaining the decreased activity or abundance of soil microorganisms associated with N cycle in some free air CO_2 enrichment (FACE) studies (Zheng et al., 2008; Kelly et al., 2013). However, other studies found that eCO_2 accelerated N transformation processes (Norby and Zak, 2011; Schleppi et al., 2012), and this was often explained by a priming effect induced by more root exudation from plants (Moser et al., 2018). Here, additional C input from root exudation can provide abundant energy for heterotrophic microorganisms, leading to increased activity of N fixation (Liu et al., 2018) and denitrification (Decock and Six, 2012) as well. Nonetheless, whether eCO_2 will increase root C inputs is still unclear, as it remains indistinct how eCO_2 alters plant C allocation strategy, including both biomass production and root exudates (Terrer et al., 2018). Apart from the effect on C and N availability, eCO_2 could also increase soil water content by decreasing water loss from transpiration with stomata of plants (Bunce, 2001), which may affect denitrification that is sensitive to soil oxygen availability (Barnard et al., 2004). Collectively, two contrasting hypotheses were proposed in the literature: i) soil denitrification will show a negative response to eCO_2 due to the decreased soil N availability (Barnard et al., 2005; Sardans et al., 2012). ii) soil denitrification will show a positive response to eCO_2 due to more soil C input in the rhizosphere via root exudation providing electron donors for denitrification (Smart et al., 1997;

Schaeffer et al., 2007) and lower O_2 availability caused by higher soil moisture (Bunce, 2001; Barnard et al., 2004) or increased soil respiration (Zhou et al., 2019; Gao et al., 2020).

Apart from the intricate mechanisms involved, the large variation may be attributed to the heterogeneity of environmental backgrounds in earlier studies. For instance, soil pH may have distinct effects on the response of soil N transformation to eCO_2 (Hall et al., 1998). In addition, short-term CO_2 enrichment may stimulate denitrification activity via additional C input increasing denitrifier abundance (Usyskin-Tonne et al., 2020), while PNL on soil denitrification may occur in the long-term exposure to eCO_2 (Tu et al., 2017). To the best of our knowledge, only two earlier meta-analyses with relatively limited data (23 and 15 data points, respectively) about the eCO_2 effects on potential denitrification were available in the literature, reporting a decrease (Barnard et al., 2005) and no significant change (Liang et al., 2016) under CO_2 elevation, respectively. To date, there is still a lack of comprehensive synthesis for the disparity of responses observed among different ecosystems and conceptual model that integrates those direct or indirect mechanisms, which calls the urgency for synthesizing and assessing the roles of these relevant factors for understanding and predicting the response of soil denitrification process to eCO_2 globally.

In this study, we provided results of a global meta-analysis to quantitatively estimate the effect of eCO_2 on soil potential denitrification rate (PDR). The aims of our study are: i) to help expand the current understanding on the impact of eCO_2 on soil PDR at the global scale; ii) to identify how the degree and duration of eCO_2, ecosystem type, and soil properties affect the response of soil PDR, and iii) to build a conceptual model illustrating how eCO_2 will influence soil denitrification process and explain the discrepancy among various experimental backgrounds. We also included data on N_2O emission and soil available N content to confirm whether they have deterministic relationships with soil PDR under eCO_2.

2 Materials and Methods

2.1 Data collection

To collect the data with the same criteria, we confined the indicator representing soil PDR to denitrification potential or denitrifying enzyme activity (Šimek et al., 2004). Those two refer to the potential N_2O-producing activity of denitrifiers in optimal conditions and are used widely as a proxy for the net denitrification rate because of the difficulty of measuring N_2 (Qin et al., 2013). To cover all studies related to the effect of eCO_2 on soil PDR published before August 2021, we used Web of Science, PubMed, and Scopus databases to search for literature with key terms of ((“elevated CO_2” OR “CO_2 enrichment”) AND (denitrif* OR (*nir*K OR *nir*S OR *nos*Z OR *nap*A OR *nar*G)) AND soil) for data of PDR and denitrifying genes abundance and ((“elevated CO_2” OR “CO_2 enrichment”) AND N_2O AND soil) for data of N_2O emission and concomitant soil available N content. Additional searching was also conducted with Google Scholar (Google, Mountain View, CA, USA) and China National Knowledge Infrastructure (CNKI, Beijing, China).

The included studies must meet the following criteria: (i) they provide the key data of ambient and eCO_2 concentration, the number of replicates and respective means of PDR, N_2O emission, soil available N content, or gene copy numbers under different CO_2 concentrations. (ii) they should also provide the key information of experimental locations (i.e., longitude and latitude) and terms to avoid duplicate records. Besides, for multifactorial experiments, we only extracted results of a single factor, CO_2 concentration, and excluded other treatments except for N addition.

We extracted data for PDR, copy numbers of genes encoding the various denitrifying enzymes, and total denitrifying bacteria abundance under ambient and eCO_2 concentration, and also extracted available data of N_2O emission in the field and soil available N (i.e., NH_4^+ & NO_3^-) content. When data was available, the ratios of *nir*K/*nos*Z, *nir*S/*nos*Z, and $N_2O/(N_2O+N_2)$ were calculated. We also extracted data of mean annual temperature (MAT, °C), mean annual precipitation (MAP, mm), experimental duration (month), CO_2 concentration (ppm), soil sampling depth (cm), soil texture, soil pH, total nitrogen (TN, g kg^{-1}), soil organic carbon (SOC, g kg^{-1}) and soil C:N ratio as possible explanatory factors. When not reported, we extracted MAT and MAP from WorldClim 2.1 database (https://www.worldclim.org/). When only the content of soil organic matter was given in the literature, we multiplied a coefficient of 0.58 to convert it to the content of SOC (Pan et al., 2004). Note that the data of the fertilization rate (g N m^{-2}) was also extracted if N addition was applied. When longitude and latitude were not directly given in the literature, we used Google Map to search them with the given names of experimental locations. We used GetData Graph Digitizer software (version 2.22) to extract the data present in figures. When a study repeatedly measured data over a short period (e.g., weekly, monthly or seasonal), we preferentially chose the measurements from the average (if available) and/or the last measurements. Finally, we included 94 studies, 432 paired observations in total across the globe (Fig. S1). Among these, 127 observations from 48 studies included data of soil PDR and denitrifying genes abundance and 305 observations from 56 studies included N_2O emission and soil available N content influenced by eCO_2. Details of the data were available in the Appendix A. Supplementary data. The process of literature survey and data screening was shown in the PRISMA guideline (Fig. S2).

According to the auxiliary data extracted from the original studies, we set different categories to conduct the subgroup analysis. The categories were shown as follows: degrees of CO_2 enrichment<50%, 50~100%, >100% (ambient: 350~450 ppm; elevated: 455~1200ppm); experimental duration<3 year, 3~7 years, 7~14 years; MAT<10 ° C, 10~15 ° C, >15 ° C; MAP<250mm (arid), 250~500mm (semi-arid), 500~800mm (semi-humid), >800mm (humid); N fertilization rate<10g m^{-2}, ≥10g m^{-2}; Soil sampling depth as 0~15cm and 15~30cm; soil texture as coarse, medium or fine; soil pH<6.5, 6.5~7.5, >7.5; SOC<20g kg^{-1}, ⩾20g kg^{-1}; TN⩽1.5g kg^{-1}, >1.5g kg^{-1}; soil C:N ratio<20, ⩾20. The C/N ratio of 20 was the equilibrium between mineralization and immobilization of soil organic matter (Brust, 2019). The soil texture was classified according to the USDA (source: http://www.nrcs.usda.gov/). We considered that studies with an experimental duration less than 3 years were divided into the short-term category. “3~7 years” and “7~14 years” were considered as middle-term studies and long-term studies, respectively. The ecosystem types we included were cropland, wetland, grassland, forest, and desert/heathland. The degree of CO_2 enrichment was calculated as (eCO_2 concentration – ambient CO_2 concentration)/ambient CO_2 concentration * 100%.

In the included studies, denitrification potential or denitrifying enzyme activity was measured by acetylene inhibition technique (AIT). The gene copy number was determined by the quantitative PCR method. The denitrifying bacteria number was determined using the most probable number method (Woomer, 1994). The N_2O emission was measured using the static gas chamber method in the field experiment.

2.2 Data process and statistical analysis

We calculated the log response ratio (lnRR) as the effect size to quantify the effect of eCO_2

by comparing means between control and treatment groups (Hedges et al., 1999). The lnRR was calculated with the equation below:

$$lnRR = \ln (X_t/X_c) \quad (1)$$

Where X_t is the mean of eCO_2 group and X_c is the mean of ambient CO_2 group.

Some studies lacked the data of standard deviation. To take full advantage of our dataset and to avoid possible extreme weights calculated by the variance-based method, we calculated the weights (w) for each observation by the equation following (Wu et al., 2021):

$$w = n_t * n_c/(n_t + n_c) \quad (2)$$

Where n_t refers to the number of replicates in eCO_2 group and n_c refers to the number of replicates in ambient CO_2 group.

We then conducted the overall effect analysis and subgroups effect analysis with the MetaWin (version 2.1) (Sinauer Associates, Inc., Sunderland, MA, USA). Data of N_2O and soil available N content was not divided into subgroups because previous studies had conducted the meta-analysis before (Liang et al., 2016; Wang et al., 2021). The variance was calculated as the reciprocal of the weight. Results were transformed to the percentage change using the equation below:

$$percentage\ change = (e^{lnRR} - 1) * 100\% \quad (3)$$

We used the random-effect model to calculate the mean effect size with lnRR. The 95% confidence intervals (CI) were generated by the bootstrap method (4999 iterations) (Rosenberg et al., 2000). We considered that the effect of eCO_2 was significant if the 95% CI did not overlap zero, and calculated between-group variations (Q_b) to determine the effects of categorical attributes. If the significance was at the level of $p<0.1$, we considered that the effect of categorical attributes was marginally significant; and if $p<0.05$, the effect of categorical attributes was considered significant. We used the weighted linear or quadratic regression to explore the potential correlations between the response ratios of soil PDR and continuous variables when they were reported simultaneously in the studies. Furthermore, we used the R package "*glmulti*" to conduct model selection analysis to quantify the importance of possible predictor variables for the effect of eCO_2 on PDR (i.e., lnRR of PDR), which was based on maximum likelihood estimation, fitting all potential models with possible predictors (Calcagno and Mazancourt, 2010). The relative importance of each predictor variable was considered as the sum of Akaike weights for all potential models, which indicated the overall predictability. We chose a cutoff of 0.8 to see if a variable was significantly predictable (Wang and Zou, 2020). To test the publication bias of our dataset, we used the R package "*metafor*" to conduct Egger's regression and draw the funnel plot (Viechtbauer, 2010). If the *p*-value of Egger's regression was more than 0.05 and the funnel plot was symmetric, we consider that there was no significant publication bias. Given that the *p*-value of Egger's regression was 0.35 and the funnel plot was generally symmetric (Fig. S3), we considered that there was no significant publication bias in our dataset.

3 Results

3.1 Effects of eCO_2 on soil PDR and other relevant indicators

Averaged across studies, we found an overall non-significant response of PDR to eCO_2 with different level concentrations (-5.2%, 95% CI: -14.4% to 5.4%) (Fig. 1a) but a significant negative response in the total abundance of denitrifying bacteria (-31.3%, 95% CI: -50.3% to -8.7%, $p<0.05$) (Fig. 1a). In contrast, eCO_2 significantly increased the abundance of *nir*S (14.7%, 95% CI:

1.2% to 32.0%, p<0.05) and *nap*A (24.4%, 95% CI: 5.5% to 44.1%, p<0.05). No significant change was found in the abundance of other denitrifying genes or ratios of key denitrifiers (*nir*K/*nos*Z or *nir*S/*nos*Z) (Fig. 1a). Concomitantly, we found a significant decrease in the content of soil NO_3^-(-15.3%, 95%CI: -22.9% to -7.5%, p<0.05), but no significant response of soil NH_4^+ under eCO_2 (Fig. 1b). Additionally, eCO_2 significantly stimulated N_2O emission by 34.1% (95%CI: 17.3% to 54.6%, p<0.05) and increased ratio of $N_2O/(N_2O+N_2)$ by 8.5% (95%CI: 1.0% to 18.0%, p<0.05) (Fig. 1b).

3.2 Bivariate relationships between effects of eCO_2 and explanatory factors

The response of PDR to eCO_2 was significantly related to climatic factors and soil properties on a global scale, particularly to the relative C and N availability. In general, we found that eCO_2 significantly decreased soil PDR when soil had a higher C availability but a lower N availability., i.e., a negative response of PDR was observed under a higher SOC content (SOC>20g kg^{-1}), lower TN content (TN≤1.5g kg^{-1}) and higher C:N ratio (C:N ratio≥20) (Fig. 2a). MAP also greatly influenced the response of PDR regarding eCO_2. According to the between-group variations (Q_b), MAP had the most significant effect on the response of soil PDR to eCO_2 (p<0.001; Table 1). Besides, we found a negative correlation between MAP and lnRR of PDR (R^2=0.032, p<0.05, n=127) (Fig. 3b). Accordingly, model selection analysis identified two predictors that exceeded the sum-of-Akaike-weights cutoff of 0.8 for all potential variables, SOC and MAP (Fig. 4). The response of PDR to eCO_2 tends to increase with the increasing soil pH, which is significantly negative when soil pH<6.5 (Fig. 2a). Furthermore, we observed a positive correlation between the degree of CO_2 enrichment and lnRR of PDR (R^2=0.060, p<0.01, n=127) (Fig. 3a). A negative response of PDR was also observed with degree of CO_2 enrichment between 50% and 100%, but we observed no significant effect with degree < 50% or >100% (slightly positive, Fig. 2b). MAT: 10~15°C, soil in grasslands and soil sampling depth between 0cm and 15cm led to a negative response of soil PDR to eCO_2 as well (Fig. 2b; Fig.S4). In addition, lnRR of PDR showed a significant quadratic relationship with MAT (R^2=0.051, p<0.05, n=127) (Fig. S5).

Concerning the lnRR of the key denitrifier community (i.e., *nir*K, *nir*S, *nos*Z), a marginal negative correlation was observed between *nos*Z and soil C:N (Fig. S6a). Besides, the response of *nos*Z to eCO_2 showed a significant correlation with N fertilization rate (p<0.001) (Fig. S6b). However, we found no significant correlations between denitrifiers and climatic factors (i.e., MAP & MAT) (Fig. S7). Note that PDR showed no significant relationship with soil available N or N_2O (Fig. S8).

4 Discussion

Denitrification removes up to 56% of newly fixed N_r at the global scale and ~8% of the terrestrial denitrification flux results in N_2O (Scheer et al., 2020), which emphasizes the importance of understanding the response of denitrification under influence of eCO_2. To provide a holistic view of denitrification under eCO_2, for the first time, we drew a conceptual diagram integrating results of our meta-analysis and mechanisms of previous studies (Fig. 5b). The lack of a significant response of overall PDR to eCO_2 shown by our results does not imply that eCO_2 has no effect on soil potential denitrification. Instead, we found that trade-offs of eCO_2 on various environmental factors, especially on soil C and N availability, with both positive and negative effects on soil PDR, contribute to the minor response of soil PDR on a global scale. The potential factors that affect the response of soil PDR to eCO_2 are discussed below in detail.

4.1 Influence factors of the response of soil PDR to eCO_2

Our results showed that the relative availability of soil N plays a predominant role in regulating soil PDR under eCO_2. The subgroup analysis in categories of soil TN, SOC, and soil C:N ratio showed that high soil C availability and low N availability would lead to significant negative responses of PDR (Fig. 2a). This fits well with our observed correlations between *nos*Z responses and soil properties or N management, thereby confirming a great sensitivity of some denitrifiers to soil C and N availability (Fig. S6). Under eCO_2, the unbalanced accumulation of C and N in ecosystems undoubtedly influences the soil C and N cycles (Luo et al., 2006). Specifically, the inadequate soil N content compared with a mass of soil C input could decrease denitrifiers activity due to the lack of N substrates (Luo et al., 2006; Singh et al., 2010). This was supported by the significant decrease of soil PDR in response to eCO_2 in grasslands (Fig. 2b), where soil often has low N availability but high content of SOC (De Klein et al., 2008). In contrast, in N-rich ecosystems like croplands, soil PDR and denitrifier abundance tend to increase with higher labile C input via root exudation (Ambus and Robertson, 1999; Lee et al., 2017). Hence, it is likely that the positive response under N-rich conditions masked the negative response under N-limiting conditions, leading to the overall observed effect size close to zero in our meta-analysis (Fig. 1a; Fig. 5b). Furthermore, ever-increasing N input often induces soil acidification in croplands and managed grasslands (Guo et al., 2010; Xu et al., 2016), thereby affecting denitrifier activity and denitrification end products (Thomas et al., 1994; Qu et al., 2014). Accordingly, we observed the negative response of soil PDR in soil with $pH<6.5$ (Fig. 2a), consistent with a microcosm experiment attributing the decrease in denitrification potential to different rhizosphere C flow at low pH (4.3) (Hall et al., 1998). Therefore, the non-significant response of soil PDR to eCO_2 on a global scale was likely partially due to the trade-off between the negative response of soil PDR caused by the PNL effect in soil and the positive response in N-rich ecosystems, which, however, may be masked by soil acidification as well.

The significant decrease (-15.3%) of soil NO_3^- content under eCO_2 suggests that eCO_2 could severely affect soil N availability (Ainsworth and Long, 2005, 2021; Norby and Zak, 2011). Notably, the decreased NO_3^- is the likely reason for decreased soil PDR in N-limiting ecosystems (Barnard et al., 2005). Whereas in N-rich ecosystems, the observed decreased NO_3^- is more likely to be the consequence of increased denitrification rate following stimulated soil PDR (Kelly et al., 2013). The contrasting responses of soil PDR under varied N availability levels may have a buffer effect on soil available N content in terrestrial ecosystems under eCO_2. That is, when N availability is relatively low (compared to that of C), soil PDR would respond negatively to eCO_2 and thus decrease ecosystem N loss; when N availability is relatively high, eCO_2 would increase soil PDR due to more labile C substrates and thus enhance the removal potential of N_r in ecosystems, thereby leading to a relatively stable content of soil available N on a continental or global spatial scale (Fig. 5a). Moreover, N availability also affects plant C investment strategy under eCO_2 (Terrer et al., 2018). Plants tend to increase allocation to root-derived C to maintain soil N availability for their growth by stimulating the soil microbiome for mineralization, which can be considered as a kind of C compensation for decreased N availability with CO_2 enrichment (Phillips et al., 2011). Whether these root-derived C would be immobilized as long-term SOC depends on plant-soil interactions and microorganisms such as denitrifiers. Further studies are needed to investigate how the interactions between plant and soil denitrification would affect N availability and C sequestration under eCO_2.

Our results suggest precipitation is an important climatic factor that affects the response of soil PDR by indirectly influencing C and N availability (Table 1; Fig. 3b; Fig. 4; Fig. S4). The negative relationship between MAP and lnRR of PDR (R^2=0.032, p<0.05) implies that the response of PDR to eCO_2 might be different under different climate conditions (Larsen et al., 2011). Previous studies showed that CO_2 enrichment stimulated plant growth more under abundant precipitation than under water deficit conditions (Billings et al., 2002; Newingham et al., 2013; Wang et al., 2019). We can infer that in moist climates where ecosystems are more likely to experience N limitation due to higher plant biomass under eCO_2, soil PDR tends to decrease due to the stronger competition for NO_3^- between denitrifiers and plants. Meanwhile, obligate heterotrophs, which can be stimulated by higher C availability in wet conditions with eCO_2, may inhibit the activity of anaerobic denitrifiers because of the competition for labile C (Billings et al., 2002). In combination, as the MAP increases, soil denitrifiers may change from water-limitation to substrate-limitation (Mueller et al., 2018), thereby affecting soil potential denitrification.

We found that the degree of CO_2 enrichment also plays a vital role in influencing the response of soil PDR. The subgroup effects and regression analysis both suggested that highly eCO_2 may increase soil PDR, while moderate eCO_2 may decrease soil PDR (Table 1; Fig. 2b; Fig. 3a). This was confirmed by a greenhouse study conducted with CO_2 concentration gradients, which found a less negative effect on denitrifier abundance under super eCO_2 (1200 μmol/mol) than under moderate eCO_2 (800 μmol/mol) (Dong et al., 2020). Distinct effects may be associated with different dominated mechanisms regarding substrate availability and aeration conditions under varying CO_2 concentrations. Moderate CO_2 enrichment may not lead to enough change in soil moisture and thus PNL is the dominant mechanism explaining decreased soil PDR (Williams et al., 2006; Qiu et al., 2019). Whereas high eCO_2 may increase soil moisture greatly and the lower O_2 content favoring higher denitrifiers activity then becomes the more dominant factor stimulating soil denitrification (Zhang et al., 2017), rather than C and N availability (Fig. 5b). Soil sampling depth is another experimental design factor that may affect the response of soil PDR due to distinct soil C availability. The different responses between the two categories (0~15cm and 15~30cm) (Fig. 2a) may be due to the different nutrient constraints. Topsoil denitrifiers with abundant C substrates are generally limited by N availability, while in deeper soil it is more controlled by C rather than N availability (Henrich and Haselwandter, 1991). In addition, some studies with diverse durations of CO_2 enrichment reported disparate responses of PDR (Rakshit et al., 2012; Moser et al., 2018; Yu et al., 2018). In our meta-analysis, however, the response of PDR showed no significant correlation with duration (data not shown) and neither were duration effects of various CO_2 enrichment levels significant (Table 1; Fig. 2b). This may be due to the contextual experimental backgrounds, i.e., site-by-site variations across the globe.

4.2 Responses of denitrifying genes abundance and N_2O emission to eCO_2

Although the effect of eCO_2 on PDR was found to be overall non-significant, we observed a significant negative response of soil denitrifying bacteria to eCO_2 (Fig. 1a). A possible explanation is the microorganism functional redundancy generated by niches overlap or phylogenic conservatism (Allison and Martiny, 2008; Jia and Whalen, 2020). Fungal denitrification, for example, was found to be stimulated under eCO_2 (Zhong et al., 2018).

However, this fungal response may be reversed when eCO_2 occurs with other possible extreme weather events such as heat waves (Bei et al., 2019). Besides, the positive response of *nar*G and *nap*A gene encoding nitrate reductase to eCO_2 possibly explains the inconsistency, as NO_3^- to NO_2^- reduction is known as the key rate-limiting step in soil denitrification. The different responses of denitrifying genes that are responsible for the same denitrification module (e.g., *nir*K & *nir*S) indicate a niche differentiation under eCO_2. Several studies reported that *nir*K- and *nir*S-harboring communities responded differently to environmental changes due to their differential niches in O_2, NO_3^- affinity, and SOC (Yuan et al., 2012; Lee and Francis, 2017). This might lead to variable shifts in community composition to acclimate to the new level of CO_2 concentration (Rosado-Porto et al., 2021, 2022).

The non-correlated relationship between N_2O emission and soil PDR indicates that denitrification is unlikely responsible for, or at least not directly associated with the higher N_2O emission under eCO_2 (Fig. S8c). Our finding does not support previous studies that attributed stimulated N_2O emission to enhanced soil denitrification under eCO_2 (van Groenigen et al., 2011; Moser et al., 2018), which may be ascribed to the difference between PDR and net denitrification rate. However, a recent ^{15}N-labeling experiment suggested that the doubling of N_2O emission observed with CO_2 enrichment was mainly due to organic N oxidation followed by incomplete NO_2^- reduction, rather than stimulated denitrification (Moser et al., 2018). Nevertheless, we found an increase in the N_2O/ (N_2+N_2O) ratio (Fig. 1b). This was often considered as a signal of incomplete denitrification producing more N_2O. Due to data paucity (n=9), this contrasting result needs to be further confirmed by more studies with more effective N_2 measuring techniques.

4.3 Implications and limitations

Our analysis identified the most probably correlating factors controlling the response of soil PDR to eCO_2 on a global scale. Besides soil properties such as C and N availability, we highlighted the role of MAP as well as the importance of experimental design, especially the degree of CO_2 enrichment. The finding that the response of soil PDR increased with the degree of CO_2 enrichment implies that the response of PDR would be dependent on the level of CO_2 increase between now and in the foreseeable future. It is worth noting that these factors collectively point to the major role of trade-offs on soil C and N availability in resulting in the minor response of soil PDR directly or indirectly, which may equilibrate the content of soil available N on a continental or global spatial scale. Our conceptual diagram illustrated how eCO_2 can influence soil potential denitrification with trade-offs in various factors, especially on soil C and N availability (Fig. 5b). This advanced the current understanding of the effect of eCO_2 on soil denitrification, which was constrained due to few experiments investigating these controlling factors synthetically and only on local scales.

We aware that our research may have some limitations. The geographical distribution of the dataset was globally restricted due to a lack of relevant studies in Africa, South America, and Russia, implying the necessity of relevant studies in these regions. Additionally, the difference between the response of potential denitrification rate and actual soil denitrification rate *in situ* must be cautiously considered. Nevertheless, as a recent perspective suggested, potential rates were still valuable when focusing on relative changes caused by environmental factors (Hazard et al., 2021). Even though global warming has been demonstrated as a consequence of eCO_2 (IPCC, 2014, 2021), elevated air temperature, a concomitant effect of eCO_2 on soil PDR, has often been neglected (Rütting and Hovenden, 2020). Also, what we are facing is a slow, but ongoing increase in atmospheric CO_2 concentration. That differs from most model simulations or field experiments, in which CO_2 concentration is increased in a short(er) time (Ollivier et al., 2011). Further empirical knowledge is therefore needed with regards to the detailed temporal response of denitrification and other N cycling processes to both temperature and CO_2 elevation. Moreover, there was a lack of study in our dataset focusing on the deeper subsoil response of PDR to eCO_2 (the max. sampling depth was 30cm). It has been proposed that eCO_2 would increase subsoil denitrification due to more labile C leaching (Harrison et al., 2008; Butterbach-Bahl and Dannenmann, 2011). Further research needs to investigate the potential response of subsoil denitrification to better estimate the full extent of ecosystem N_r removal. Furthermore, it was reported that highly eCO_2 (1000~30000ppm) can directly deteriorate the denitrification through decreasing the production of cellular NADH and denitrifying gene abundance, which, however, was mostly neglected (Wan et al., 2018).

In summary, our study found a minor effect of elevated CO_2 on soil potential denitrification rate on a global scale, which is likely due to trade-offs on soil carbon and nitrogen availability under elevated CO_2. Our findings provide significant implications for biogeochemical models to predict terrestrial N cycle in future scenarios with elevated CO_2. Further field studies concerning integrating elevated CO_2 with various climatic and edaphic factors are highly warranted.

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Appendix A. supplementary data

Supplementary materials to this article, including original data for this meta-analysis, can be found in the online version.

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Table 1. Between-group variability (Q_b) of different category variables among observations (n) suggesting their effects as predictive variables influencing soil potential denitrification responses to eCO2. Statistical significance of Q_b: †*p* < 0.1; ***p*<0.05, ****p*<0.01, *****p*<0.001.

Table 1.

Variables	n	Q_b
All observations	127	-
MAP	127	17.167***
MAT	127	3.412
Ecosystem type	127	5.172
Degree of CO_2 enrichment	127	4.855†

Experimental duration	115	1.677
N fertilizer type	40	0.032
N fertilizer rate	66	0.255
Soil sampling depth	125	1.224
SOC	43	1.278*****
TN	48	3.219*****
C:N	31	0.143
Soil pH	77	2.028
Soil texture	120	0.899

Fig caption

Fig. 1. The overall effect of elevated CO_2 (eCO_2) on **(a)** potential denitrification rate (PDR) and denitrifying gene abundance, **(b)** N_2O emission, the ratio of N_2O/N_2O+N_2 and the content of soil available N. Mean values, 95% CI ($p<0.05$) and the number of observations for each indicator are shown in the figure. To judge the significance better, the vertical dashed line is drawn to mark the effect size of zero.

Fig. 2. The overall effect and comparisons for each category of elevated CO_2 (eCO_2) on potential denitrification rate (PDR) with subgroups for **(a)** soil properties and **(b)** other explanatory variables including experimental design, ecosystem types, and N fertilization. Mean values, 95% CI ($p<0.05$) and the number of observations for each category are shown in the figure. Categorical

variables are given on the right side of the figure. "$p<0.05$" and "$p<0.1$" are shown with according categorical variables if the effects of categorical attributes are significant or marginally significant, respectively. The vertical and horizontal dashed lines are drawn to judge the significance and to distinguish different categorical variables, respectively.

Fig. 3. Relationships between the lnRR of potential denitrification rate (PDR) and **(a)** degree of elevated CO_2 (eCO_2), **(b)** mean annual precipitation (MAP). Various colors represent different ecosystem types. Black lines represent the fitted linear regressions with standard error (gray shading) and data points were weighted based on the replicates of each observation. The plots also show values representing fitting degree (R^2), significance level (p), and the number of sampling size for regression analysis (n).

Fig. 4. Results of model selection analysis from R package "*glmulti*". The length of the pillar represents the averaged importance of the predictive variable, which is calculated by the sum of Akaike weights. The vertical line shows a cutoff of 0.8 to distinguish important predictors.

Fig. 5. Conceptual diagrams illustrating potential mechanisms regarding soil PDR under elevated CO_2. **(a)** A conceptual diagram illustrating how the trade-off or negative feedback loop on the response of soil PDR to elevated CO_2 can equilibrate the N availability level (green). As the level of N availability increases, soil PDR responds to elevated CO_2 from negatively (blue) to positively (red), leading to a relatively stable content of soil available N. **(b)** A conceptual diagram illustrating how elevated CO_2 (eCO_2) can influence soil denitrification. Red and blue boxes or arrows represent mechanisms or experimental factors that have positive and negative effects on the response of soil denitrification to eCO_2, respectively. The green box represents the microbial-driven process of soil denitrification and the main results of this meta-analysis. Green dashed

arrows indicate that eCO_2 had a minor (~) and indirect effect on soil potential denitrification rate (PDR) due to trade-offs on soil C and N availability. Upward and downward arrows (↑, ↓) refer to the increase and decrease of according factors or variables, respectively. Arrows with dashed lines and solid lines refer to indirect and direct effects, respectively. Solid boxes and dotted boxes represent mechanisms from previous studies and influencing factors concluded from this study, respectively. Abbreviation: MAP, mean annual precipitation; PNL, progressive N limitation.

Fig. 1.

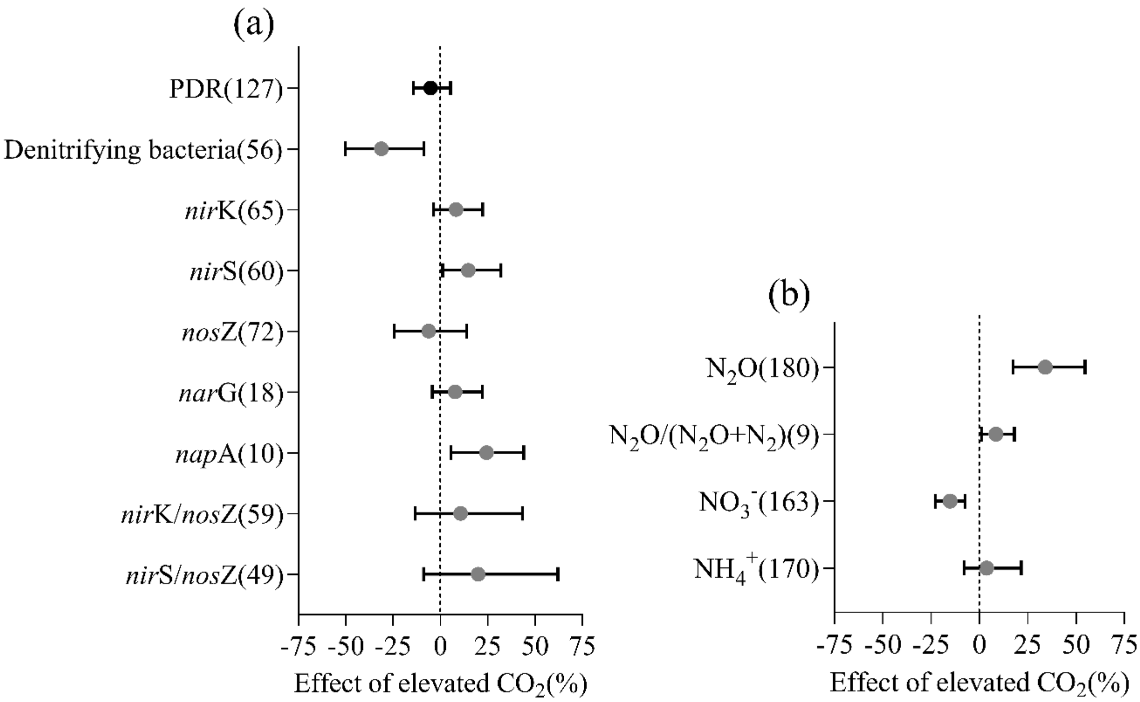


Fig. 2.

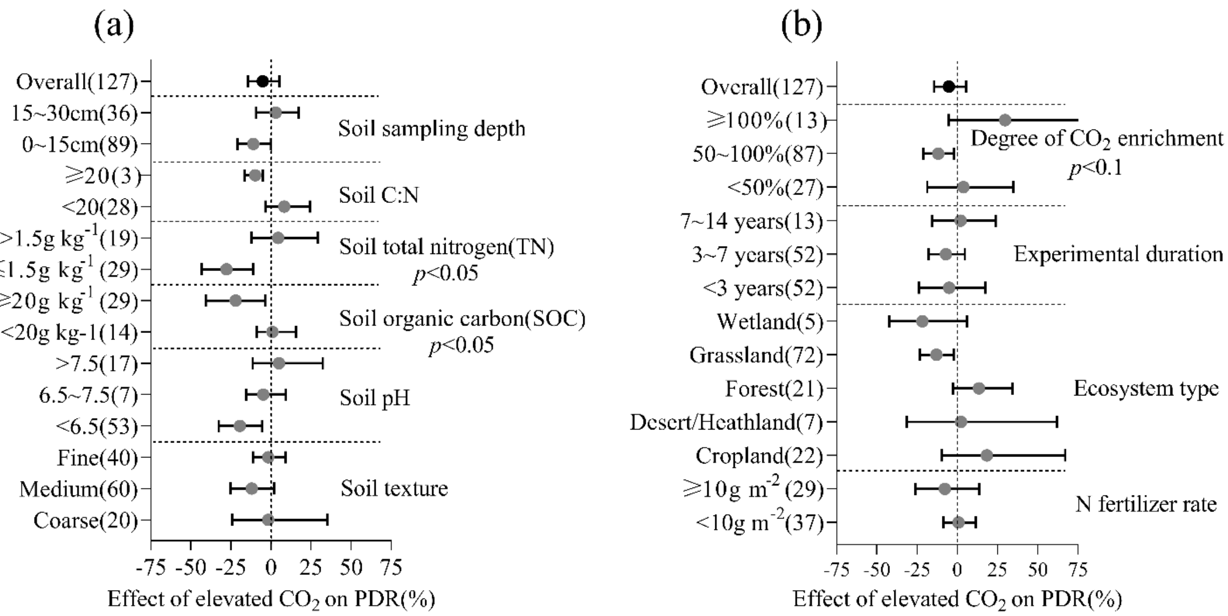

(a)
Overall(127)
15~30cm(36)
0~15cm(89)
Soil sampling depth
⩾20(3)
<20(28)
Soil C:N
>1.5g kg-1(19)
⩽1.5g kg-1 (29)
Soil total nitrogen(TN)
p<0.05
⩾20g kg-1 (29)
<20g kg-1(14)
Soil organic carbon(SOC)
p<0.05
>7.5(17)
6.5~7.5(7)
<6.5(53)
Soil pH
Fine(40)
Medium(60)
Coarse(20)
Soil texture
-75 -50 -25 0 25 50 75
Effect of elevated CO2 on PDR(%)
(b)
Overall(127)
⩾100%(13)
50~100%(87)
<50%(27)
Degree of CO2 enrichment
p<0.1
7~14 years(13)
3~7 years(52)
<3 years(52)
Experimental duration
Wetland(5)
Grassland(72)
Forest(21)
Desert/Heathland(7)
Cropland(22)
Ecosystem type
⩾10g m-2 (29)
<10g m-2(37)
N fertilizer rate
-75 -50 -25 0 25 50 75
Effect of elevated CO2 on PDR(%)

Fig. 3.

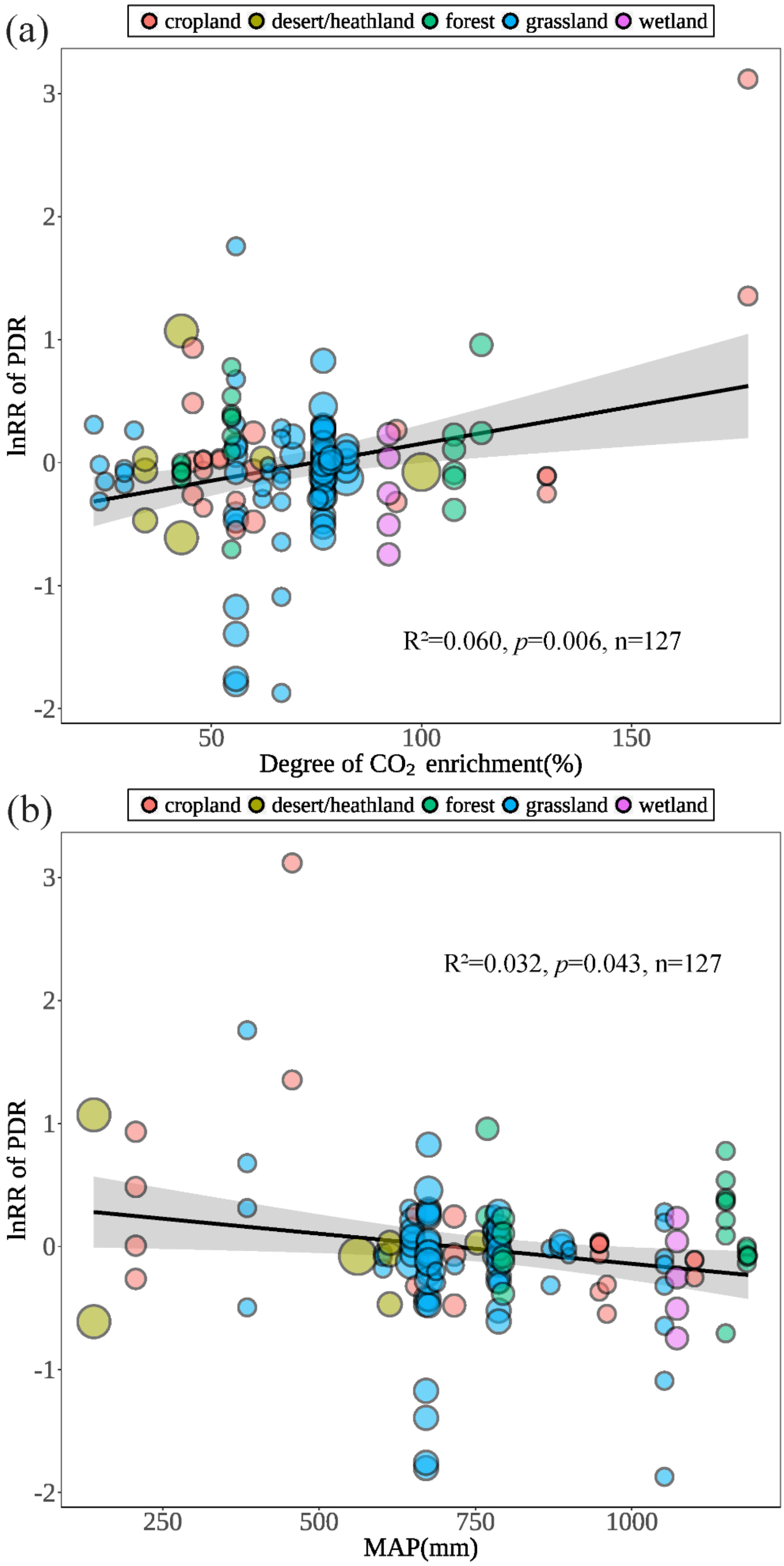

(a)
cropland desert/heathland forest grassland wetland
lnRR of PDR
3
2
1
0
-1
-2
R²=0.060, p=0.006, n=127
50
100
150
Degree of CO₂ enrichment(%)
(b)
cropland desert/heathland forest grassland wetland
lnRR of PDR
3
2
1
0
-1
-2
R²=0.032, p=0.043, n=127
250
500
750
1000
MAP(mm)

Fig. 4.

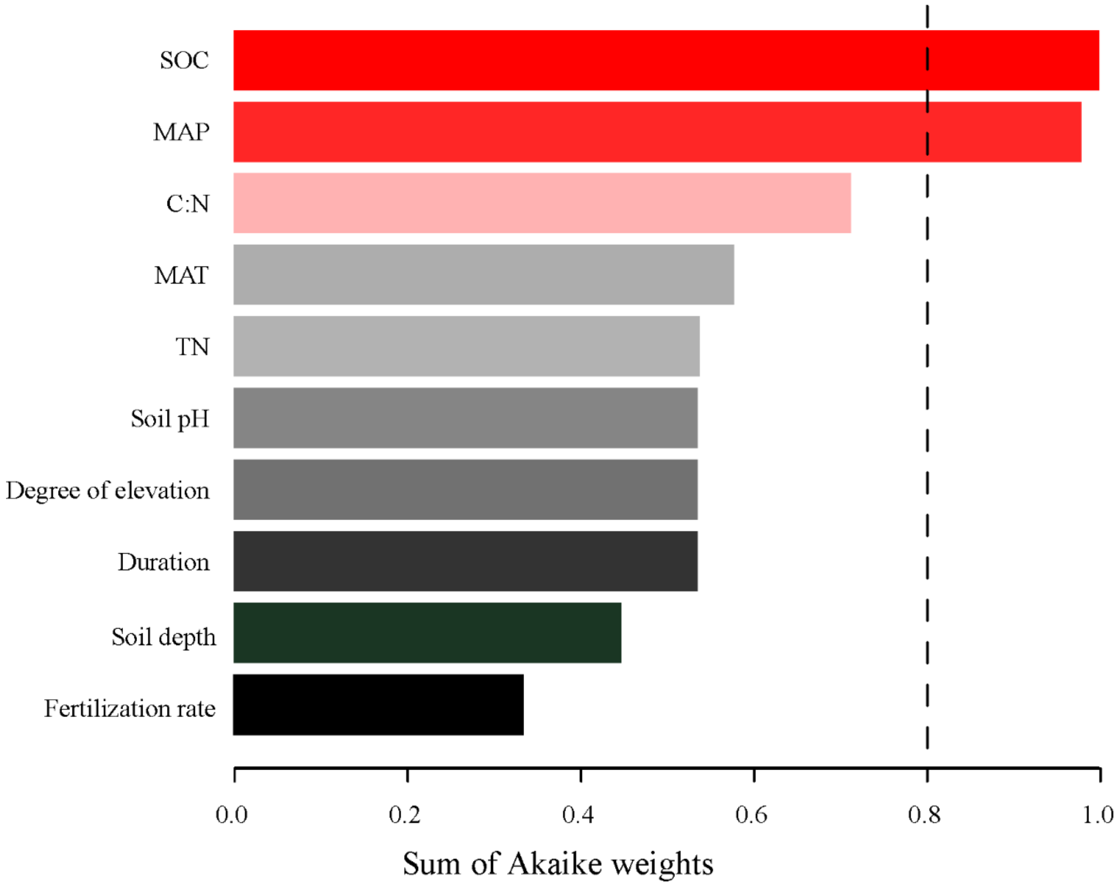
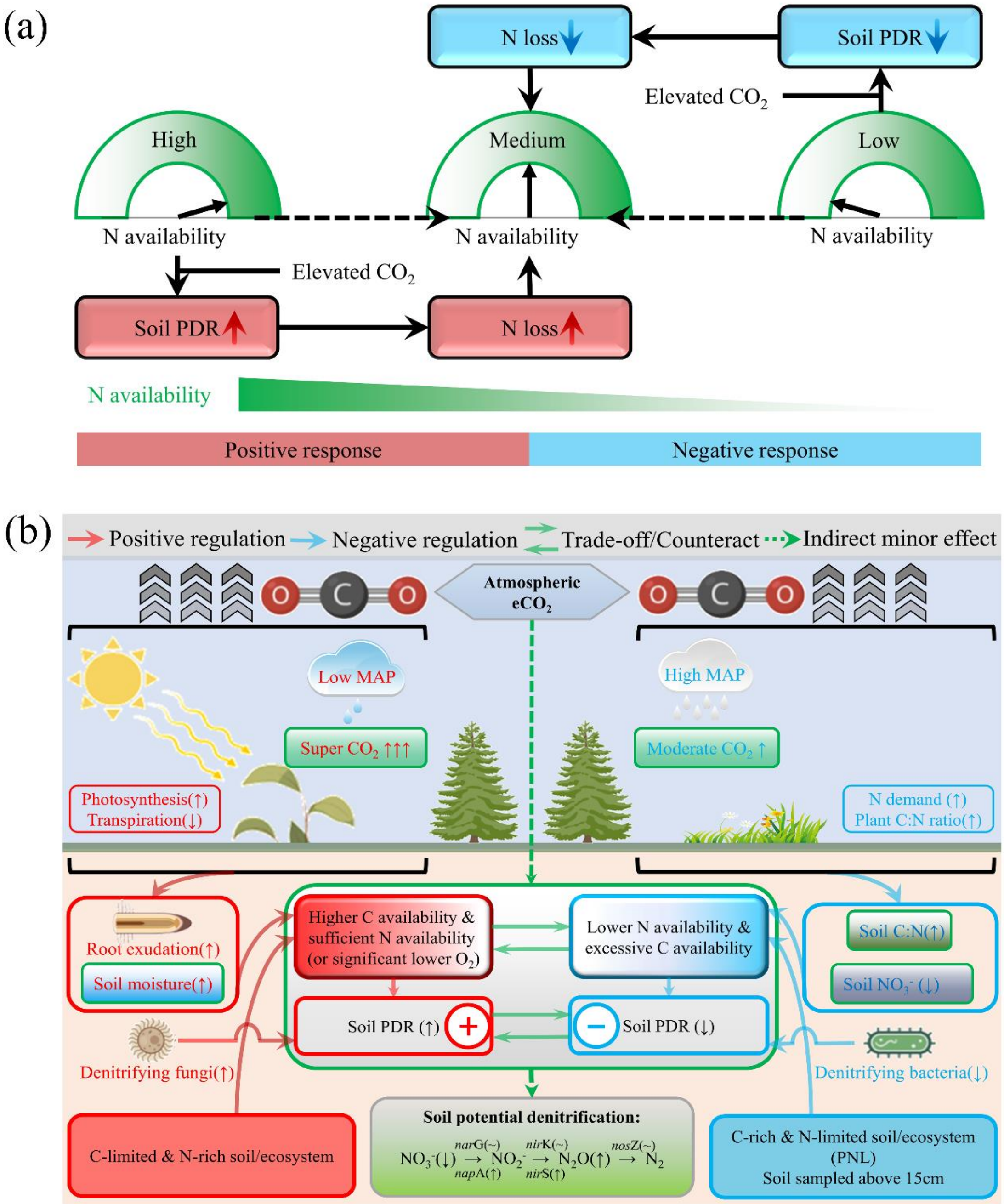

SOC
MAP
C:N
MAT
TN
Soil pH
Degree of elevation
Duration
Soil depth
Fertilization rate
0.0
0.2
0.4
0.6
0.8
1.0
Sum of Akaike weights

Fig. 5.


(a)
N loss
Soil PDR
Elevated CO2
High
Medium
Low
N availability
N availability
N availability
Elevated CO2
Soil PDR
N loss
N availability
Positive response
Negative response
(b)
Positive regulation
Negative regulation
Trade-off/Counteract
Indirect minor effect
Atmospheric eCO2
Low MAP
High MAP
Super CO2 ↑↑↑
Moderate CO2 ↑
Photosynthesis(↑)
Transpiration(↓)
N demand (↑)
Plant C:N ratio(↑)
Root exudation(↑)
Soil moisture(↑)
Higher C availability & sufficient N availability (or significant lower O2)
Lower N availability & excessive C availability
Soil C:N(↑)
Soil NO3- (↓)
Soil PDR (↑)
Soil PDR (↓)
Denitrifying fungi(↑)
Denitrifying bacteria(↓)
C-limited & N-rich soil/ecosystem
Soil potential denitrification:
NO3-(↓) → NO2- → N2O(↑) → N2
narG(~) napA(↑) nirK(~) nirS(↑) nosZ(~)
C-rich & N-limited soil/ecosystem
(PNL)
Soil sampled above 15cm