Responses of nitrogen concentrations and pools to multiple environmental change drivers: a meta-analysis across terrestrial ecosystems

Running head: Terrestrial N response to environmental change

Abstract

Aim: We sought to understand how the individual and combined effects of multiple environmental change drivers differentially influence terrestrial nitrogen (N) concentrations and N pools and whether the interactive effects of these drivers are mainly antagonistic, synergistic or additive.

Location: Worldwide.

Time period: Contemporary.

Major taxa studied: Plant, soil, and soil microbes in terrestrial ecosystems.

Methods: We synthesized data from manipulative field studies from 760 published articles to estimate the individual, combined, and interactive effects of key environmental change drivers (elevated CO_2, warming, N addition, phosphorus addition, increased rainfall, and drought) on plant, soil and soil microbe N concentrations and pools using meta-analyses. We assessed the influences of moderator variables on these effects through structural equation modeling.

Results: We found that (1) N concentrations and N pools were significantly affected by the individual and combined effects of multiple drivers, with N addition (either alone or in combination with another driver) showing the strongest positive effects; (2) the individual and combined effects of these drivers differed significantly between N concentrations and N pools in plants, but seldom in soils and microbes; (3) additive effects of driver pairs on N concentrations and pools were much more common than synergistic or antagonistic effects across plants, soils, and microbes; and (4) environmental and experimental factors were important moderators of the individual, combined, and interactive effects of these drivers on terrestrial N.

Main conclusions: Our results indicate that terrestrial N concentrations and N pools, especially those of plants, can be significantly affected by the individual and combined effects of environmental change drivers, with the interactive effects of these drivers being mostly additive. Our findings are important because they contribute to the development of models to better predict how altered N availability affects ecosystem carbon cycling under future environmental changes.

Keywords: altered rainfall, combined effects, elevated CO_2, individual effects, interactive effects, nitrogen addition, phosphorus addition, warming

1 Introduction

Human-induced changes in the biogeochemical cycling of key nutrient elements such as nitrogen (N) can strongly influence ecosystem structure and function (Gruber & Galloway, 2008). Changes in N availability can remarkably affect multiple biological processes, including plant photosynthesis, symbiotic N fixation, and microbial N mineralization, with potential consequences for terrestrial carbon (C) cycling and storage (Peñuelas et al., 2013; Vitousek, 2004; Yue et al., 2016). Enhanced N availability across terrestrial ecosystems has been occurring with simultaneous alterations in other environmental change drivers, including elevated atmospheric CO_2 (eCO_2), warming, increased rainfall (rainfall$^+$), and drought, which can have divergent effects (Galloway, 2005; Peñuelas et al., 2013; Sardans et al., 2017). For example, recent meta-analyses reported that N enrichment can significantly increase plant and soil N concentrations (Lu et al., 2011), whereas eCO_2 (Deng et al., 2015) and drought (He & Dijkstra, 2014) can have the opposite effect on plant

N concentrations. Despite our growing understanding of the individual effects that different environmental change drivers may have on terrestrial N concentrations, few attempts have been made to address whether and how such individual effects on N concentrations (percent of dry mass) and N pools (absolute mass per unit area) may differ significantly across different terrestrial compartments (i.e., plants, soils, and soil microbes). How these environmental change drivers interact to affect N concentrations and N pools in different terrestrial ecosystem compartments also remains unclear.

Changes in N concentrations and N pools in response to environmental change drivers are likely to vary strongly among (and within) plants, soils, and microbial biomass (Lu et al., 2011; Sardans et al., 2017). For example, the negative effect of eCO_2 on plant N concentration (Cotrufo, Ineson, & Scott, 1998) does not necessarily indicate a reduction in the total amount of N in plants (i.e., the plant N pool) because eCO_2 could simultaneously stimulate plant biomass production (Nowak, Ellsworth, & Smith, 2004). Likewise, the negative effects of drought on plant N concentration can be reversed when plant biomass proportionally decreases more than plant N uptake under drought stress (He & Dijkstra, 2014). Human-induced phosphorus (P) fertilization also significantly influences plant N concentration (Yuan & Chen, 2015), while N-P imbalances following repeated P fertilization can alter the functions of both natural and managed ecosystems (Peñuelas et al., 2013). These findings suggest the need to disentangle plant N concentration and N pool responses from different environmental change drivers. Soil or microbial N concentrations and pools also vary in response to environmental change drivers. For example, it has been shown that N fertilization can significantly influence soil bulk density by mediating the activities of soil fauna and microbes (Crill, Martikainen, Nykanen, & Silvola, 1994), and that the application of N alone or in combination with other nutrients (e.g., P) can affect soil N pools differently (Fornara, Banin, & Crawley, 2013). Although previous synthesis studies (Bai et al., 2013; Lu et al., 2011) have addressed the potential responses of terrestrial N pools to multiple drivers, these studies have not distinguished between N concentrations and N pools and have mainly focused on the effects of individual drivers.

Multiple environmental change drivers likely act simultaneously and influence a wide range of ecological and biogeochemical processes (Reich et al., 2006; Yue, Fornara, Yang, Peng, Peng, et al., 2017); thus, the combined effects of multiple drivers on N cycling may be more important than the corresponding individual effects. For instance, a recent meta-analysis (Li, Niu, & Yu, 2016) showed how the combined effect of N and P additions on plant N concentration tends to be higher than their individual effects. On the other hand, the positive effects of eCO_2 or N addition on plant N concentration may be suppressed by the presence of another driver, such as drought (Delgado-Baquerizo et al., 2013). Additionally, reductions in soil N concentrations under eCO_2 can be nullified by simultaneous warming (Hovenden et al., 2008). Although warming can significantly increase both plant and soil N pools (Bai et al., 2013), it can also promote drought stress, which makes predictions of the N pool response to warming more complex and uncertain.

Another important knowledge gap is whether the interactive effects of multiple environmental change drivers on terrestrial N concentrations and pools are additive or non-additive. Additive interactions occur when the combined effect of two or more drivers is equal to or not significantly different from the sum of the individual effects; otherwise, the interaction is either synergistic or antagonistic (Jessica Gurevitch, Morrison, & Hedges, 2000; Zhou et al., 2016). Both additive and nonadditive effects have been found in empirical and synthesis studies. For example, the interactive effects of eCO_2 and warming on plant biomass were found to be nonadditive in a grassland manipulation experiment (Mueller et al., 2016), which agrees with a meta-analysis across different ecosystem types (Dieleman et al., 2012). However, findings from other recent syntheses showed how eCO_2, warming, N addition, P addition, and altered rainfall regimes generally result in additive interactions between individual drivers for plant N concentrations, plant C:N:P stoichiometry, and terrestrial C and P pools (Yuan & Chen, 2015; Yue, Fornara, Yang, Peng, Li, et al., 2017; Yue, Fornara, Yang, Peng, Peng, et al., 2017; Yue et al., 2018). These studies further suggested that the interactive effects of multiple environmental change drivers may vary substantially depending on the tested variables and combinations of drivers.

Here, we first compiled data from 760 published articles reporting field manipulative experiments in both natural and managed ecosystems and then adopted a meta-analysis approach to explicitly compare effect sizes of individual, combined and interactive effects of eCO_2, warming, N addition, P addition, rainfall$^+$ and drought on plant N, soil N [including total N, inorganic N, ammonium (NH_4^+), and nitrate (NO_3^-)] and soil microbial biomass N (MBN) concentrations and pools. Finally, we used structural equation models (SEMs) to assess the role of several environmental and experimental factors (i.e., moderator variables) in influencing the individual, combined, and interactive effects of different environmental change drivers. The main objectives of this study were to (1) quantify the individual and combined effects of multiple drivers on N concentrations and pools in different ecosystem compartments (plants, soils and microbial biomass), (2) assess whether the individual or combined effects of multiple drivers on N concentrations and pools differ, and (3) evaluate whether the interactive effects of these drivers on N concentrations or pools are additive.

2 Materials and methods

2.1 Data extraction and compilation

The *ISI Web of Science*, *PubMed,* and *Google Scholar* were used to search for peer-reviewed journal articles published before 31 January 2018. We focused on field manipulative studies that included the key environmental change drivers eCO_2, warming, N addition, P addition, rainfall$^+$, and drought as well as any combination of these six drivers. The criteria for inclusion in our database included the following: (1) manipulative experiments were conducted in the field to collect data regarding at least one of the studied drivers; (2) experimental and control plots were established within the same ecosystem and contrasted in terms of only the target variable; (3) the magnitude of the treatment and the study duration were clearly recorded, and measurements of the variables in the experimental and control groups were performed at the same spatial and temporal scales; (4) the duration of the manipulative experiments was no less than one growing season; and (5) the means, sample sizes, and standard deviations (SD) or standard errors (SE) of the chosen variables were directly provided or could be estimated from the reported data.

Plant N concentrations at both the species and community levels were directly recorded from the primary studies, and N pools were either directly recorded or calculated as the product of N concentration and plant biomass. Because most of the primary studies reported data from only mineral soil layers, we considered only the mineral soil layer in this meta-analysis. Data for soil and microbial biomass N concentrations were extracted from the primary studies, while N pools were directly extracted or determined based on the soil bulk density (if available), microbial biomass, and the corresponding N concentrations. In addition, plant biomass data at both the species and community levels were collected only when simultaneous data for N concentrations or pools were reported. When several measurements were taken at different times in a single primary study, we used values from the last measurement to meet the statistical assumption of independence among observations in the meta-analysis (Hedges, Gurevitch, & Curtis, 1999).

Furthermore, as the observations from a single primary study representing different plant compartments, ecosystem types and/or climates may not be independent, we used mixed-effects models and treated studies as random factors in the analysis (Koricheva, Gurevitch, & Mengersen, 2013). Because the number of studies assessing the combined effects of three or more drivers was too small for a meta-analysis, we considered only driver pairs in this study. When the data in primary studies were presented graphically, the figures were digitized to extract the numerical values using the free software Engauge Digitizer, version 5.1 (Free Software Foundation, Inc., Boston, MA, USA). Climate variables [i.e., mean annual temperature (MAT) and mean annual precipitation (MAP)] were obtained directly from the primary studies or extracted from *WorldClim* version 2.0 (http://www.worldclim.org) using location information in the cases in which these data had not been reported.

After extraction, the data from 760 published articles, representing 7622 observations from all continents except Antarctica, were included in our database (see Text S1, Table S1, and Fig. S1 in Appendix 1). In this meta-analysis, we investigated the individual effects of each of the studied environmental change drivers, the combined effects of each driver pair (denoted driver 1 + driver 2) and the interactions between each driver pair (denoted driver 1 × driver 2, see Fig. 1a, b). To calculate the first two types of effects, we used the natural-log response ratio (lnRR). We used lnRR because it shows the least bias of the commonly used effect size metrics and its sampling distribution approximates normality (Hedges et al., 1999). In addition, as we want to know the proportional changes in the response variables relative to controls, lnRR is easily interpretable. Interactive effects between two drivers could be calculated from only studies with a full factorial design (see Fig. 1a). Given that few studies met this criterion, we used Hedges' *d* to assess interaction effects because it is an estimate of the standardized mean difference that is not biased by small sample sizes (Gurevitch & Hedges, 2001).

2.2 Analysis of individual and combined effects

The effect of one environmental change driver or the combined effect of a driver pair was defined as the response of a variable (e.g., soil N concentration) in a treated sample compared with the value of that variable in the corresponding control (Yue, Fornara, Yang, Peng, Peng, et al., 2017), and was described by lnRR (Hedges et al. 1999). The calculations of lnRR, the associated variance (v_1) and weight (w_1) of each lnRR, and the weighted mean lnRR ($lnRR_{++}$) are described in detail in Text S2 of Appendix 1. The individual or combined effect was not significant ($P < 0.05$) if the 95% confidence interval (CI) of $lnRR_{++}$ overlapped with zero (Rosenberg, Adams, & Gurevitch, 2000). We used the equation $\left(e^{\mathrm{lnRR}_{++}} - 1\right) \times 100\%$ to calculate the net responses of N concentrations or pools to the individual or combined effects in terms of the mean percentage of change relative to the control value (%), and the effects were considered not significant at the $p < 0.05$ level if the 95% CI overlapped with zero. The $lnRR_{++}$ and associated 95% CI values were calculated using mixed-effect models in MetaWin 2.1 (Rosenberg et al., 2000).

Several environmental and experimental factors may influence the individual, combined, and interactive effects on terrestrial N and were thus included in the meta-analysis as moderator variables (Koricheva et al., 2013). We categorized the constructed database into different subgroups according to ecosystem type (boreal forest, temperate forest, subtropical and tropical forest, grassland, wetland, tundra, shrubland, desert, and cropland), plant functional type (woody and herbaceous), treatment magnitude, type of manipulative facility [open-top chamber (OTC), free-air CO_2 enrichment (FACE), and screen-aided CO_2 control (SACC) for eCO_2; OTC and heater for warming], and fertilizer chemical form (NH_4NO_3, NH_4, NO_3, urea, and mixture of NH_4NO_3

and urea) to assess the influence of these categorical moderator variables on effect size. The effect of each categorical moderator on lnRR was evaluated by comparing the heterogeneity within (Q_w) and between (Q_b) moderator levels using mixed-effect models in MetaWin 2.1 (Borenstein, Hedges, Higgins, & Rothstein, 2009). To assess the influence of the continuous moderator variables latitude, MAT, MAP, treatment magnitude, and study duration, an *a priori* conceptual SEM (Fig. 1c) was developed based on current ecological knowledge (Grace, 2006). This SEM was tested separately for each driver or driver pair. We examined the distributions of the endogenous and exogenous variables of the SEM analysis, tested their normality and transformed them when necessary. The covariance between duration and magnitude was included in the model. The analysis was conducted only when the number of data points was > 30, and we used a bootstrapping method for resampling based on 5000 iterations when the number of data points was < 100 (Grace, 2006). The overall goodness-of-fit of the models was tested using the traditional χ^2 goodness-of-fit test and the root mean square error of approximation (RMSEA) index (Grace, 2006; Schermelleh-Engel, Moosbrugger, & Müller, 2003). The SEM analyses were performed with AMOS software version 22.0 (Amos Development Co.).

2.3 Analysis of interactive effects

To further assess whether interactive effects were additive, we employed Hedges' *d* according to established methods (Gurevitch et al., 2000). Accordingly, the interaction effect size (d_I) between drivers A and B was calculated by Eqn (1):

$$d_I = \frac{(X_{AB} - X_A) - (X_B - X_C)}{s} J(m) \qquad (1)$$

where X_C, X_A, X_B, and X_{AB} are the means of a variable in the control, the treatment groups A and B, and their combination group (AB), respectively. The variables *s* and *J(m)* are the pooled SD and a correction term for small sample sizes, respectively, which were calculated by Eqns (2) and (3).

$$s = \sqrt{\frac{(n_c - 1)s_c^2 + (n_A - 1)s_A^2 + (n_B - 1)s_B^2 + (n_{AB} - 1)s_{AB}^2}{n_c + n_A + n_B + n_{AB} - 4}} \qquad (2)$$

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

where n_C, n_A, n_B, and n_{AB} are the corresponding sample sizes; s_C, s_A, s_B, and s_{AB} are the SDs in the control and experimental groups of A, B, and their combination (AB), respectively; and m is the degree of freedom ($m = n_c + n_A + n_B + n_{AB} - 4$). The variance in d_I (v) was estimated by Eqn (4):

$$v = \frac{1}{n_c} + \frac{1}{n_A} + \frac{1}{n_B} + \frac{1}{n_{AB}} + \frac{d_I^2}{2(n_c + n_A + n_B + n_{AB})} \qquad (4)$$

The weighted mean d_I (d_{++}) was calculated according to Eqn (5):

$$d_{++} = \frac{\sum_{i=1}^{l}\sum_{j=1}^{k} w_{ij} d_{ij}}{\sum_{i=1}^{l}\sum_{j=1}^{k} w_{ij}} \qquad (5)$$

where l is the number of groups, k is the number of comparisons in the i^{th} group, w is the study weight [which is also the reciprocal of the variances ($1/v$)] and d is the size of the individual effect. The 95% CI of d_{++} was calculated as $d_{++} \pm C_{\alpha/2} \times s(d_{++})$, where $C_{\alpha/2}$ is the two-tailed critical value of the standard normal distribution. When the number of data points was < 20, we used a bootstrapping method for resampling to obtain the 2.5% lowest and highest values as CIs based on 5000 iterations (Janssens et al., 2010; Zhou et al., 2016). The interactions between two drivers were classified as additive, synergistic, or antagonistic (Jessica Gurevitch et al., 2000; Zhou et al., 2016). An interactive effect was considered additive if the 95% CI overlapped with zero. If the individual effects of driver pairs were either both negative or have opposite directions, the interactions whose total effects were less than 0 were synergistic, and those whose total effects were greater than 0 were antagonistic. When the individual effects were both positive, the interactions were interpreted conversely (i.e., those >–0 were synergistic, and those <–0 were antagonistic).

3 Results

3.1 Individual effects of environmental change drivers on terrestrial N concentrations and pools

The responses of plant N concentrations and pools to individual drivers differed significantly ($p < 0.001$) at both species and community levels (Fig. 2a). Specifically, eCO_2 significantly decreased plant N concentrations by 7% at both species and community levels, but had no effect or a significantly positive (6%) effect on plant N pools at species and community levels. Warming significantly increased plant N pool at species level by an average of 11%, and N addition stimulated plant N concentrations and pools at both species and community levels. P addition significantly increased plant N concentration by 6% at the community level and plant N pools at species (19%) and community levels (24%). Rainfall$^+$ showed significant effects on plant N concentration (5%) at the species level. Drought had no effect on plant N concentration or N pools. The responses of plant biomass to individual drivers were generally significantly correlated with the responses of the corresponding plant N pools at both species and community levels, but were not correlated with plant N concentration (Table 1). Soil N concentration significantly increased by 10% and 6% under N and P additions, respectively, while soil N pool was significantly enhanced only by N addition (12%, Fig. 2b). The concentration and pool of soil inorganic nitrogen (SIN) significantly decreased by 11% and 34%, respectively, under eCO_2, but significantly increased by 14% and 19% under warming and by 79% and 125% under N addition. The SIN, soil NH_4^+ and soil NO_3^- were rarely affected by the individual drivers (Fig. S2). In addition, MBN concentration significantly increased by 8% under eCO_2, while MBN pool increased by 17% under N addition.

3.2 Combined and interactive effects of environmental change drivers on terrestrial N concentrations and pools

The combined effects of N addition + eCO_2, N addition + warming, N addition + P addition, and N addition + rainfall$^+$ significantly increased plant N concentration at species level by 4%, 50%, 13% and 29%, respectively. Their effects on the corresponding N pool were significantly higher, with increases of 44%, 223%, 89%, and 226%, respectively (Fig. 3a). In contrast, eCO_2 + warming significantly decreased plant N concentration at the species level by 10%, but had no effects on plant N pool at species level. Both plant N concentration and N pool at community level were stimulated significantly by N addition + P addition, with an average increase of 16% and 66%, respectively. In addition, the responses of plant biomass and N pool to the combined effects of environmental change drivers showed significant positive correlations (Table 1).

There was no significant difference between N concentration and N pool in relation to the combined effects of environmental change drivers for soil or microbial biomass (Fig. 3b). Soil total N concentration significantly increased by 6% under warming + N addition and N addition + P addition, while soil total N pool was significantly stimulated (12%) only by N addition + P addition. The combined drivers eCO_2 + N addition, warming + N addition, warming + drought, and N addition + rainfall$^+$ significantly increased SIN by 36%, 97%, 49%, and 61%, respectively, but had no effect on SIN pool. Soil NO_3^- concentration and pool responded similarly to the SIN, while NH_4^+ concentration was enhanced only by eCO_2 + N addition (+39%) or N addition + P addition (+45%) (Fig. S3). However, neither the concentration nor the pool of MBN was affected by the combined effects (Fig. 3c).

In terms of the interactive effects, additive effects on terrestrial N concentrations and pools were more frequently found than synergistic and antagonistic effects across all the driver pairs tested in our study (Fig. 4). With the exception of an antagonistic effect of N addition × rainfall$^+$, the interactive effects of other driver pairs on plant N concentration at the species level were all additive (Fig. 4a). The mean interactive effect of N addition × P addition on plant N concentration at the community level was antagonistic, but their effect on plant N pool at both the species and community level was synergistic. All interactive effects on the concentrations and pools of soil total N and SIN were additive except for that of N addition × P addition on SIN concentration, which was synergistic. Likewise, similar patterns were found for NH_4^+ and NO_3^- (Fig. S4), and the interactive effects of all driver pairs on MBN concentrations and pools were additive (Fig. 4). Moreover, despite the observation of several overall non-additive interactive effects, the frequency distribution of interaction types indicates that additive interactions were substantially predominant across all the driver pairs (Fig. 4).

3.3 Influences of moderator variables

Moderator variables such as ecosystem type, experimental design factors (e.g., study duration, facility, treatment magnitude, and fertilizer form), latitude, and climate (i.e., MAT and MAP) all mediated the responses of terrestrial N concentrations and pools to individual, combined, and interactive effects of the investigated drivers (Fig. 5 and Fig. S5-S13 in Appendix 2). For example, the individual effects of eCO_2 on plant N concentration (Q_b = 15.25, P = 0.033) and pool (Q_b = 18.50, P = 0.010) varied significantly with ecosystem type (Fig. S5a) and were also significantly influenced by the magnitudes of eCO_2, MAP, and study duration (Fig. S13a). Likewise, N addition

+ P addition on plant N concentration and pool significantly varied with ecosystem type (Fig. S18c, d) and were significantly influenced by latitude, MAT, and MAP in some cases (Fig. 5a), although such impacts on N concentrations and pools vary with terrestrial compartment. The effect of eCO_2 × warming on plant N concentrations was significantly influenced by latitude and MAP, while N addition × P addition on plant N pools at both species and community levels were significantly modulated by experiment duration (Fig. 5b).

4 Discussion

Our results show how N concentrations and N pools in plants, soils, and microbial biomass are influenced by the individual and combined effects of different environmental change drivers. We found that N concentrations and pools in plants show higher sensitivity to these driver effects than those in soils and microbial biomass. The different responses between plant N concentrations and N pools to the individual or combined effects of multiple drivers suggest that mixing plant N concentration and N pool data can be problematic when plant N cycling is assessed under different environments. However, such a difference was seldom observed in soils or microbial biomass. In addition, our results show that the interactive effects of multiple drivers on N concentrations and N pools of plants, soils and microbes are more likely to be additive. These novel findings contribute to improving our understanding of how terrestrial N cycling among plants, soils, and microbes may shift under the simultaneous effects of multiple environmental change drivers.

4.1 Differential individual effects of environmental change drivers on terrestrial N concentrations and pools

The magnitude and direction of environmental change effects on terrestrial N vary depending on the identity of the driver and the nature of the N concentration or pool (i.e., plants *vs*. soils *vs*. soil microbial biomass). For example, our results show how eCO_2 significantly decreased plant N concentration at both species and community levels, but significantly increased plant N pool at the community level (Fig. 2a). Net negative effects of eCO_2 on plant N concentration may occur because of (1) dilution of N by increased photosynthetic assimilation of C; (2) lower transpiration-driven mass flow of N in soils due to decreased stomatal conductance under eCO_2; and (3) increased rates of N loss via volatilization and/or root exudation, which may further decrease tissue N concentration (Taub & Wang, 2008). The increase in plant N pools under eCO_2 could be related to eCO_2-induced increases in biological N fixation or increases in root growth for N uptake (Luo et al., 2004). Warming-induced net N accumulation in plants (i.e., plant N pool) may be attributed to enhanced plant growth that is being stimulated by changes in soil N availability (Vitousek & Howarth, 1991) and in plant phenology (Luo, Sherry, Zhou, & Wan, 2009) under warming conditions. This idea was supported by our results showing that SIN concentration and pool significantly increased in the warming treatments (see Fig. 2a).

Nitrogen addition generally showed positive effects on plant N concentrations and N pools (Fig. 2a) and on soil N pools (Fig. 2b). These findings agree with the results of previous meta-analyses, which showed that N addition had positive effects on plant N concentrations and N pools (Bai et al., 2013; Lu et al., 2011). The significant positive effects that P addition and rainfall^{+} had on plant N concentrations and pools (Fig. 2a) may be attributed to the fact that both P and water availability are limiting factors for plant growth and N uptake (Li et al., 2016). In contrast, drought showed minimal effects on plant, soil and microbial biomass N concentrations and pools. Microbial biomass N pools were significantly increased by eCO_2 (Fig. 2c). Previous studies showed that soil microbial numbers, metabolic activity, and biomass can be increased by eCO_2

(Sadowsky & Schortemeyer, 1997). Thus, this increase can be attributed to eCO_2 increasing the microbial utilization of soil organic matter (Carney, Hungate, Drake, & Megonigal, 2007) and N fixation ability through stimulating the activities of related enzymes (Cheng et al., 2011; He et al., 2010).

Our results show that the responses of plant N concentrations and N pools to the individual effects of multiple drivers can vary significantly (Fig. 2a), suggesting that the effects on these two variables should be separately tested when plant N dynamics are assessed in future studies. We found that while the responses of plant biomass to these drivers were significantly correlated with the corresponding plant N pools, they were not related to plant N concentrations (Table 1). This phenomenon is likely to occur when one driver or driver pair increases plant biomass without changing N concentration, thus resulting in an increase in the N pool (Doiron, Gauthier, & Lévesque, 2014). Our evidence is that the responses of soil N and MBN to the individual drivers are weaker than plant N responses (Fig. 2), which may partly occur because soil N pools are much larger than plant N pools (Nieder & Benbi, 2008) and thus expected to respond more slowly to environmental change. In addition, changes in soil N pools may also be difficult to detect because of the higher inherent variation in soil organic matter under environmental change.

4.2 Combined effects and the interactions of driver pairs

Our results showed that eCO_2 + N addition, eCO_2 + drought, and warming + N addition stimulated plant N concentrations and N pools at the species level (Fig. 3a). The positive effects of eCO_2 + N addition on plant N concentrations and N pools may be attributed to the fact that additional N input meets the increased plant N demand under eCO_2 (Reich, Hobbie, & Lee, 2014). The stimulating effects of eCO_2 + drought on plant N pools were mainly attributed to the net positive effect of

eCO_2 because, as shown in our results, drought had no effect on plant N pools and the interactive effect of eCO_2 × drought was additive.

Plant growth and plant biomass production in terrestrial ecosystems are primarily limited by N availability or are co-limited by P availability (Elser et al., 2007; Vitousek, Porder, Houlton, & Chadwick, 2010). Larger inputs of one nutrient (either N or P) will stimulate plant growth but also lead to increased demand for the other nutrient. Thus, the simultaneous addition of N and P can significantly contribute to increasing plant N uptake rates, which can lead to increased N concentrations and N pools (Li et al., 2016). N addition × P addition on plant N pools was indeed synergistic at both species and community levels, albeit antagonistic for plant N concentrations at community level (Fig. 4). N addition increases the demand for P by stimulating plant growth; thus, extra P addition could counterbalance this N-induced P limitation, allowing the full N fertilization effect to be expressed (You et al., 2018). In this case, the N addition effect could be larger in the combined treatment with P than in the N-only treatment, resulting in a synergistic N addition × P addition effect. However, the occurrence of such a synergistic effect is conditional, as the interactive effect was significantly influenced by experimental and environmental factors such as latitude and study duration (see Fig. 5b). The full expression of the N addition effect with additional P input can be conditional because net N addition effects on plant N uptake are soil dependent and thus are influenced by multiple biogeochemical factors (Niu et al., 2016). When increases in plant biomass are larger than increases in N uptake, N addition × P addition effects on plant N concentration could be antagonistic but could become synergistic for N pools as a result of increases in N mass. However, despite these non-additive effects, our results indicated that additive effects were much more common across individual observations and that the overall effect of N addition × P addition on plant N concentrations at the species level was additive.

We found that N addition + $rainfall^+$ had significant positive effects on plant N concentrations and N pools at the species level (Fig. 3a) and that the mean interactive effect of N addition × $rainfall^+$ on plant N concentrations was antagonistic (Fig. 4). Water availability is an important factor regulating ecosystem primary production, and increased water input generally stimulates biomass production through increased uptake of limiting nutrients such as N (Li, Lin, Taube, Pan, & Dittert, 2011). However, although N addition can enhance plant N uptake, it can also decrease plant N use efficiency (Lü, Dijkstra, Kong, Wang, & Han, 2014); thus, the interaction of N with $rainfall^+$ can be antagonistic for plant N concentrations. Nevertheless, the additive interactions of N addition × $rainfall^+$ on plant N concentrations remained predominant at the species level.

Soil total N concentration was significantly enhanced by warming + N addition and N addition + P addition (Fig. 3b). Although N addition alone significantly increased soil N concentration, further positive effects on soil total N occurred when the N fertilization effect (i.e., N-induced increases in plant N input to soils) was enhanced by warming or P addition (Bai et al., 2013). Because the SIN pool is smaller than the soil total N pool (Benbi & Richter, 2003), the variations in SIN can be more sensitive than the variations in the soil total N pool to environmental change drivers, as we found in this study (Fig. 3b). Common additive interactions were also observed for soil total N and SIN, although we observed an overall synergistic effect of N addition × P addition on SIN concentration (Fig. 4). N addition can increase phosphatase activity and thus soil P availability (Olander & Vitousek, 2000), but this N-induced potential increase in P availability is usually insufficient to balance the accompanying increased P limitation. Thus, the simultaneous addition of P and N would enhance SIN because P addition can also stimulate rates of N fixation and increase N availability (Crews, Farrington, & Vitousek, 2000). Hence, the synergistic effects of N addition × P addition on SIN concentration are not unrealistic. In addition, the overall synergistic effect on SIN could result from the large weight that individual synergistic observations may have relative to more common (> 50%) additive effects (Zhou et al., 2016). We observed similar patterns of additive interactive effects on MBN concentrations. However, in contrast to plant and soil N, soil MBN exhibited no significant response to the combined effects of driver pairs, which may be attributed to the small sample size that limited the breadth of our analysis (Loladze, 2014).

4.3 Moderating effects of environmental and experimental factors

Moderator variables, such as environmental and experimental factors, which affect the individual effects of environmental change drivers on specific ecosystem properties, have been highlighted and discussed in previous studies (Bai et al., 2013; Li et al., 2016; Lu et al., 2011; Xia & Wan, 2008). Similarly, our results revealed that such moderator variables significantly influence the combined and interactive effects of driver pairs on plant and soil N concentrations and pools. For example, the combined effects of eCO_2 + N addition on plant N concentrations and pools significantly varied with ecosystem type, with significantly positive effects in grassland, but no effects in tropical forests. This difference may be because eCO_2-induced nutrient limitations were related to not only N, but also other nutrients such as P (Winter, Garcia, Gottsberger, & Popp, 2001), whose availability is typically limited in tropical forests (Elser et al., 2007). The interactive effects of eCO_2 × N addition on plant N were also significantly affected by moderator variables such as latitude and MAP (Fig. 5b). As discussed above, driver effects are expected to be environmentally dependent. Confirming this expectation, our results suggest that MAP influences the extent to which driver effects are expressed and modulates the interactive effects of driver pairs on N cycling. Likewise, we found that the effects of N addition + P addition and N addition × P addition on the concentrations and pools of plant and soil N were significantly modulated by latitude, MAT, MAP, and experimental duration (Fig. 5b, S13). This relationship could be attributed to ecosystem N cycling differing along gradients generated by these environmental and experimental factors (Bai et al., 2013; Lu et al., 2011). Moreover, the effect size of N addition + P addition on soil NO_3^- concentration was significantly positively correlated with the duration of the manipulation study (Fig. S13), indicating that long studies are necessary to completely assess environmental change effects on terrestrial N pools.

4.4 Uncertainty analysis and limitations

Although our meta-analysis provides new evidence of how the individual, combined, and interactive effects of multiple environmental change drivers might influence terrestrial N concentrations and N pools, significant uncertainty still remains. First, primary studies assessing the effects of environmental changes on plants, soils, and microbes are not abundant enough and unequally distributed geographically, thus limiting our ability to conduct a global analysis of N cycling among different ecosystem components. Evaluating combined effects is even more challenging because the small sample sizes of data for many driver pairs hampered our ability to quantify the nature of the interactions. Additionally, the lack of data for other driver pairs and potential combinations of three or more drivers reduced the breadth of our analysis. Second, published studies on global change effects on nitrogen dynamics are strongly biased towards the northern hemisphere (see Fig. S1); thus, the database failed to represent ecosystem types equally at the global scale, especially savanna and tropical forest ecosystems. Third, in terms of statistics, the observation-weighted approach that we used here might overestimate the amount of additive interactions associated with large variance in some observations (Gurevitch et al., 2000; Zhou et al., 2016). Nevertheless, our statistical analysis showed that the average weights of the interaction (d_I) for significant results (synergistic and antagonistic effects) were similar to those for the non-significant interactions (additive effects) (see Table S3). This result suggests that overestimation of additive interactions should not be an issue (Zhou et al., 2016).

4.5 Implications and perspectives

Our study shows how plant N concentrations and N pools were differently affected by the individual and combined effects of multiple environmental change drivers, indicating that merging these two N variables would potentially be misleading when assessing the response of plant N dynamics to environmental change. The weak responses of soil and microbial N concentrations and N pools (compared to plants) to both individual and combined effects of multiple drivers suggest that soils and soil microbes are less sensitive than plants to environmental change or that their responses are more difficult to detect. Most importantly, our study suggests that responses of N concentrations and N pools in different terrestrial compartments to the interactive effects of multiple drivers are more likely to be additive than synergistic or antagonistic. These common additive effects of driver pairs on N concentrations and pools should be incorporated into ecosystem models that aim to predict how altered N availability affects global C sinks. Future studies could address what underlying biogeochemical mechanisms enhance the stability of soils and soil microbes to environmental change. Finally, well-designed long-term experiments that simultaneously assess effects of multiple drivers on ecosystem compartments are urgently needed to better capture the dynamics of terrestrial N cycling and their consequences for terrestrial C storage under future environmental change scenarios.

Data accessibility

All the data used in the meta-analysis are included in the Supporting Information.

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Supporting Information

Appendix 1 Data sources and methods.

Appendix 2 Supporting results.

Table 1 Pearson correlation coefficients (r) between the effect sizes of global change driver (lnRR) on plant biomass and terrestrial N concentrations (-C) and pools (-P).

Terrestrial N	eCO_2	Warming	N addition	P addition	Rainfall^{+}	Drought	eCO_2 + N	N + P	N + rainfall^{+}	eCO_2 + warming
PN-C (species)	-0.102 (136)	0.152 (46)	0.054 (203)	-0.104 (51)	-0.085 (11)	0.184 (10)	0.372 (15)	-0.255 (25)	-0.028 (9)	-0.476 (13)
PN-P (species)		0.801^{***} (43)	0.925^{***} (216)	0.942^{***} (45)	0.907^{***} (11)	0.697^{*} (10)	0.989^{***} (15)	0.960^{***} (35)	0.969^{***} (9)	0.609^{*} (9)
PN-C (community)	-0.405 (11)	0.002 (8)	-0.254^{*} (92)	-0.064 (50)				-0.130 (45)		
PN-P (community)	0.971^{***} (15)	0.843^{***} (15)	0.608^{***} (93)	0.873^{***} (44)		0.880^{***} (12)		0.797^{***} (42)		
SN-C		< 0.001 (13)	-0.099 (105)	-0.042 (22)				-0.216 (30)		
SN-P		0.046 (30)	0.078 (56)					-0.036 (14)		
SIN-C		0.227 (11)	0.148 (59)	-0.037 (23)				-0.113 (27)		
SIN-P			0.166 (27)							
NH_4^+-C		0.385 (14)	0.205 (205)	0.130 (17)				-0.192 (20)		
NH_4^+-P		0.617 (10)	0.609 (8)					-0.327 (9)		
NO_3^--C		0.444 (13)	-0.058 (54)	-0.061 (17)				-0.318 (20)		
NO_3^--P			0.750^{*} (8)					-0.009 (9)		
MBN-C			-0.654^{**} (17)							
MBN-P		0.155 (23)								

Values in brackets indicate the sample size of observations. Correlation analysis was conducted only when the number of data points was > 8. Asterisks indicate significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) correlations. PN: plant nitrogen; SN: soil total nitrogen; SIN: soil inorganic nitrogen; MBN: microbial biomass nitrogen; eCO_2: elevated CO_2; rainfall^{+}: increased rainfall.

Figure captions

Figure 1 (a) Three common experimental designs included in our database to study the effects of two environmental change drivers, A and B, with C being the control plot. Individual effects of drivers A and B were calculated based on data from study designs 2 and 3. The combined effects, A+B, were calculated based on data from designs 1 and 3, while the interactive effects, A × B, could be calculated from only study design 3 (i.e., A+B = A + B + A × B). (b) A real picture of the Cedar Creek Ecosystem Science Reserve in Minnesota, US. Several experiments at this research site examined the interactions between multiple environmental drivers [Photo credit: Jacob Miller, 2014, CC BY-SA 4.0]. (c) An *a priori* conceptual structural equation model (SEM) depicting the influence of latitude, longitude, mean annual temperature (MAT), mean annual precipitation (MAP), driver magnitude, and study duration on the effect size (lnRR or d_I) of environmental change drivers on terrestrial N concentrations or pools. The same model was used for all tested drivers and driver pairs. Single-headed arrows indicate a hypothesized directional influence of one variable on another, double-headed arrows represent a correlation in which no direction is specified, and each square indicates a measured variable entered in the model. Note that "magnitude" was tested for only individual drivers and the combination N addition + P addition, in which case the ratio between the added N and P (N:P) was used.

Figure 2 Individual effects of multiple environmental change drivers on terrestrial N concentrations and pools in (a) plants, (b) soils and (c) soil microbial biomass. The results are expressed as the percentage change relative to the control (%). Values indicate the means with 95% confidence intervals (CIs), and sample size numbers for N concentrations and pools are shown in parentheses. The effects of environmental change drivers are significant when the 95% CIs do not overlap with zero. The results are not presented when the sample size is < 3. Asterisks indicate significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) differences between the responses of N concentrations and pools to a specific driver. eCO_2: elevated CO_2; N: nitrogen addition; P: phosphorus addition; $Rainfall^{+}$: increased rainfall.

Figure 3 Combined effects of multiple environmental change drivers on terrestrial N concentrations and pools in (a) plants, (b) soils and (c) soil microbial biomass. The results are expressed as the percentage change relative to the control (%). Values indicate the means with 95% confidence intervals (CIs), and sample size numbers for N concentrations and pools are shown in parentheses. The effects of environmental change drivers are significant when the 95% CIs do not overlap with zero. The results are not presented when the sample size is < 3. Asterisks indicate significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$) differences between the responses of N concentrations and pools to a specific driver pair. eCO_2: elevated CO_2; N: nitrogen addition; P: phosphorus addition; $Rainfall^{+}$: increased rainfall; MBN: microbial biomass nitrogen.

Figure 4 Interactive effects of multiple environmental change drivers on terrestrial (a) N concentrations and (b) N pools and the corresponding frequency distribution of interaction types among individual observations of driver pairs. Dots represent means with 95% confidence intervals (CIs), and sample size numbers are shown in parentheses. If the 95% CI overlapped with zero, the interactive effect was considered to be additive; otherwise, the interactive effect was synergistic or antagonistic. The results are not presented when the sample size is < 3. Because many studies reported only combined effects, sample sizes may be smaller than the corresponding ones in Fig. 3. Values in percentages indicate the proportions of additive interactions among all the individual observations of a specific response variable. PN: plant nitrogen; SN: soil total nitrogen; SIN: soil inorganic nitrogen; MBN: microbial biomass nitrogen; eCO_2: elevated CO_2; N: nitrogen addition; P: phosphorus addition; Rainfall: increased rainfall.

Figure 5 Standardized total effects (direct plus indirect effects) derived from structural equation models (SEMs) evaluating the influence of moderator variables (depicted in different colors) on (a) the combined effects (lnRR) and (b) interactive effects (d_I) of driver pairs on terrestrial N concentrations (-C) and pools (-P). Note that the magnitude of the tested driver pairs was assessed in only N and P combinations, where the ratio between the added N and P (N:P ratio) is used. Asterisks indicate significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{**}p < 0.001$) direct effects. See Table S2 in Appendix 2 for the goodness-of-fit tests of the SEMs, and see Fig. S13 in Appendix 2 for the results derived from the SEMs evaluating the influence of moderator variables on the individual effects. eCO_2: elevated CO_2; PN: plant nitrogen; SN: soil total nitrogen; SIN: soil inorganic nitrogen; MBN: microbial biomass nitrogen.

Figure 1

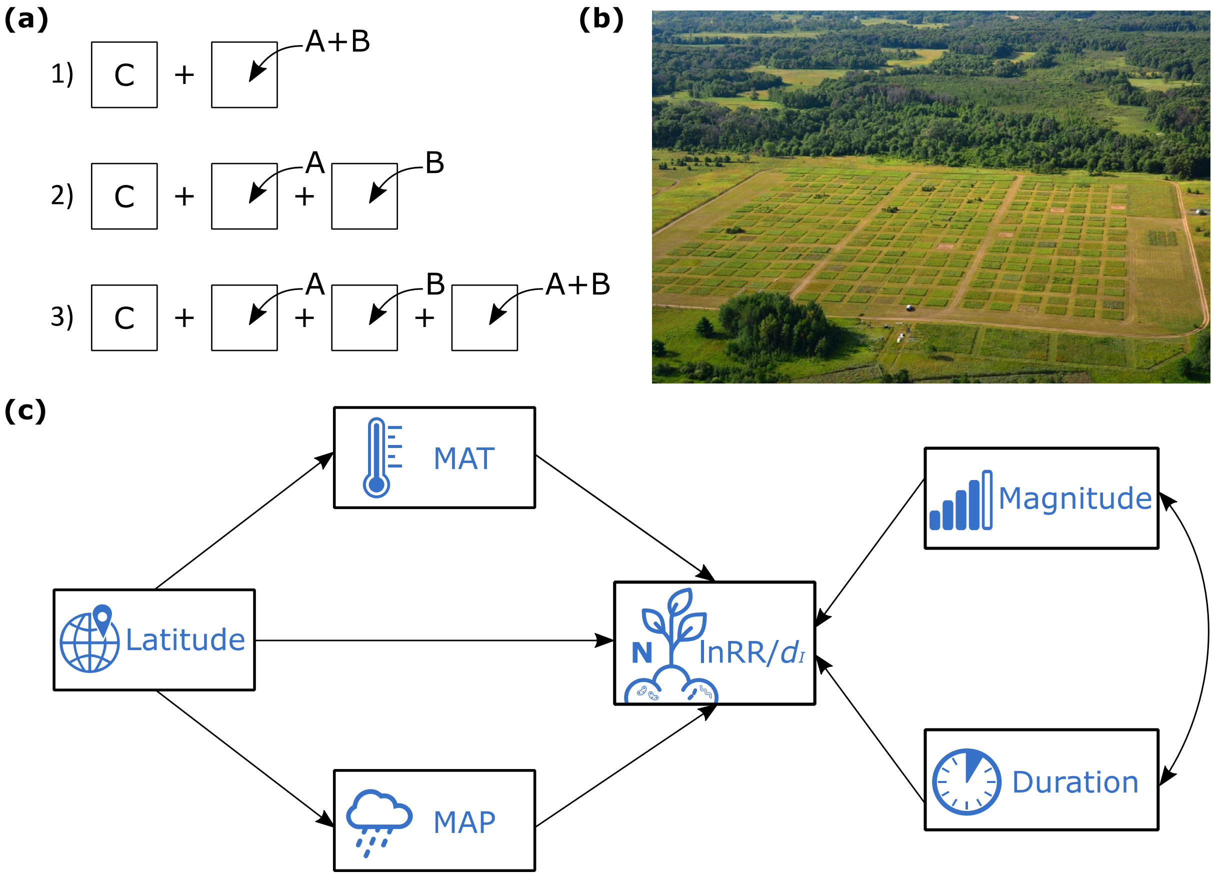

(a)
1) C + A+B
2) C + A + B
3) C + A + B + A+B
(b)
(c)
MAT
Latitude
MAP
N
lnRR/dI
Magnitude
Duration

Figure 2

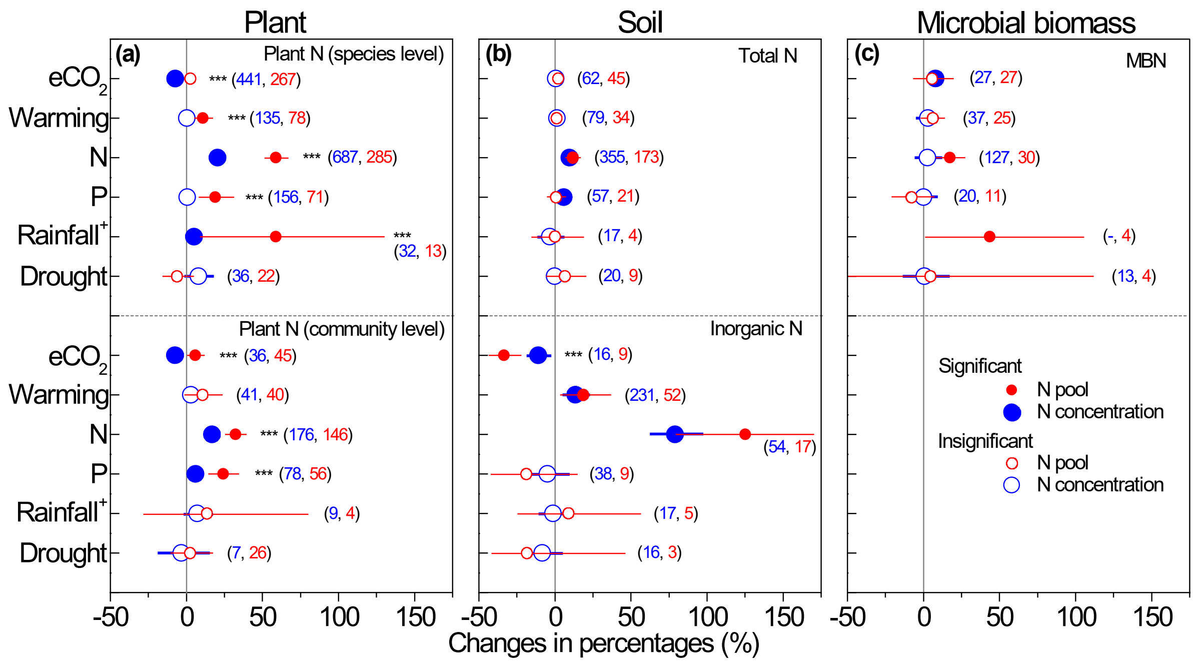

Plant
Soil
Microbial biomass
(a)
Plant N (species level)
(b)
Total N
(c)
MBN
eCO2
Warming
N
P
Rainfall+
Drought
*** (441, 267)
*** (135, 78)
*** (687, 285)
*** (156, 71)
***
(32, 13)
(36, 22)
(62, 45)
(79, 34)
(355, 173)
(57, 21)
(17, 4)
(20, 9)
(27, 27)
(37, 25)
(127, 30)
(20, 11)
(-, 4)
(13, 4)
Plant N (community level)
Inorganic N
*** (36, 45)
(41, 40)
*** (176, 146)
*** (78, 56)
(9, 4)
(7, 26)
*** (16, 9)
(231, 52)
(54, 17)
(38, 9)
(17, 5)
(16, 3)
Significant
N pool
N concentration
Insignificant
N pool
N concentration
-50
0
50
100
150
Changes in percentages (%)

Figure 3

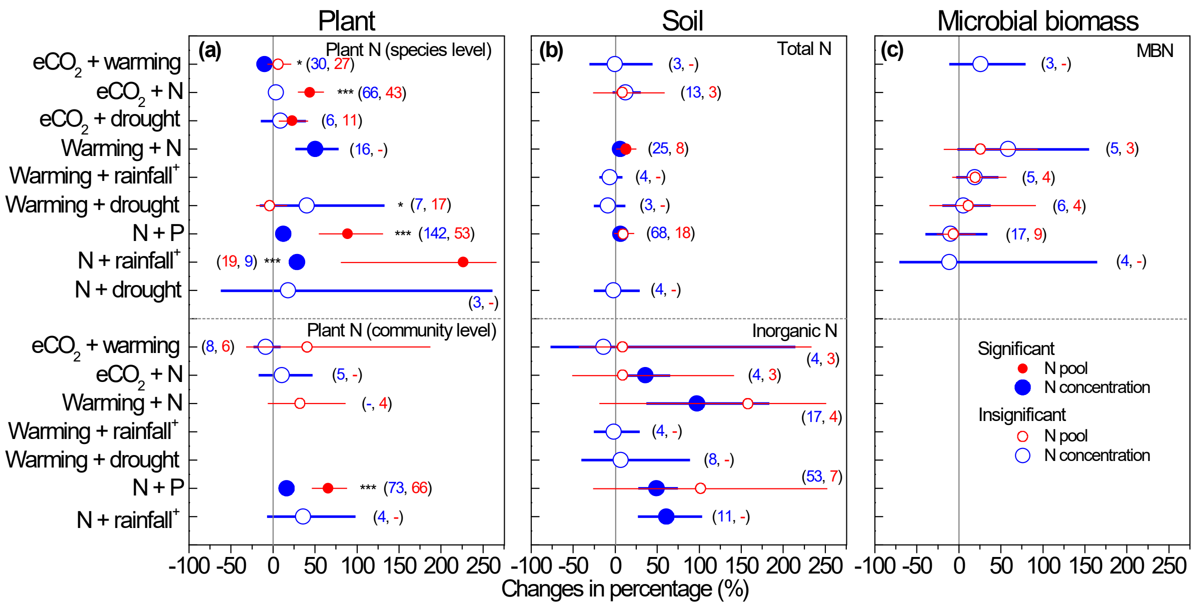
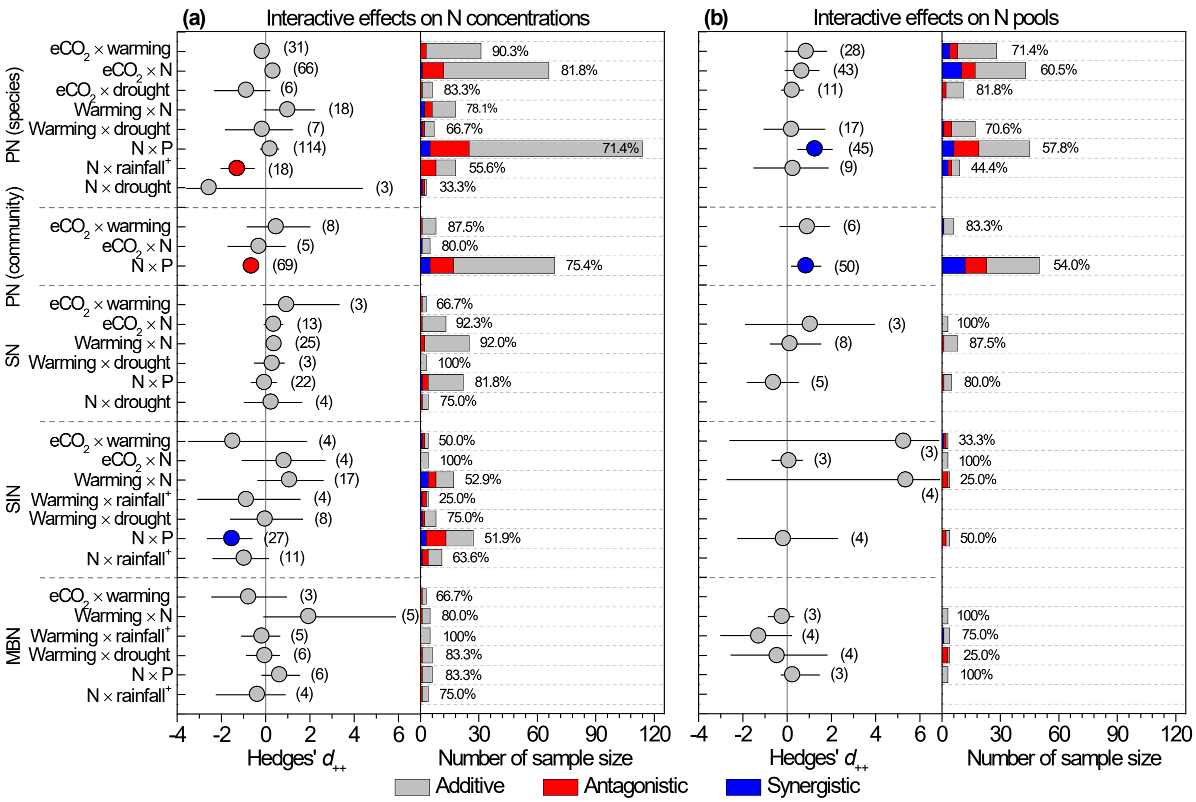
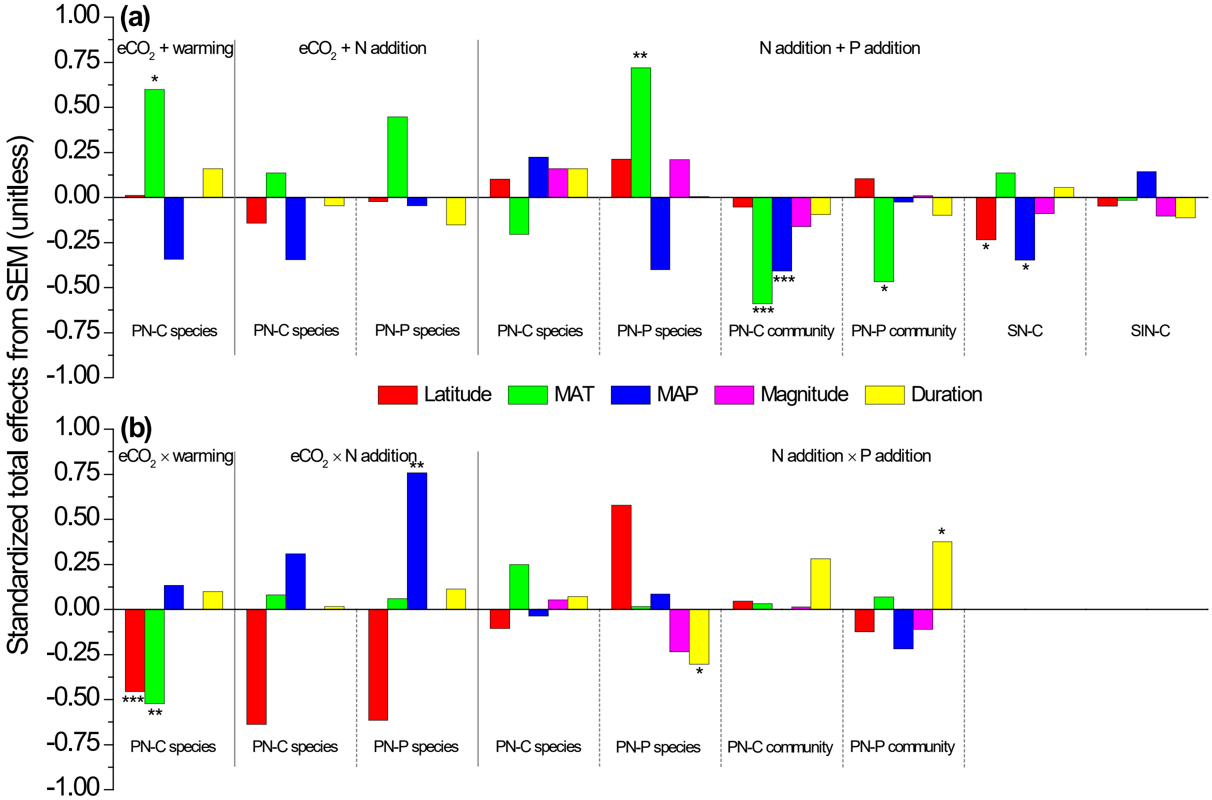

Plant
Soil
Microbial biomass
(a)
Plant N (species level)
(b)
Total N
(c)
MBN
eCO2 + warming
eCO2 + N
eCO2 + drought
Warming + N
Warming + rainfall+
Warming + drought
N + P
N + rainfall+
N + drought
* (30, 27)
*** (66, 43)
(6, 11)
(16, -)
* (7, 17)
*** (142, 53)
(19, 9) ***
(3, -)
(3, -)
(13, 3)
(25, 8)
(4, -)
(3, -)
(68, 18)
(4, -)
(3, -)
(5, 3)
(5, 4)
(6, 4)
(17, 9)
(4, -)
Plant N (community level)
Inorganic N
eCO2 + warming
eCO2 + N
Warming + N
Warming + rainfall+
Warming + drought
N + P
N + rainfall+
(8, 6)
(5, -)
(-, 4)
*** (73, 66)
(4, -)
(4, 3)
(4, 3)
(17, 4)
(4, -)
(8, -)
(53, 7)
(11, -)
Significant
N pool
N concentration
Insignificant
N pool
N concentration
-100 -50 0 50 100 150 200 250
Changes in percentage (%)

Figure 4



(a)
Interactive effects on N concentrations
(b)
Interactive effects on N pools
PN (species)
PN (community)
SN
SIN
MBN
eCO2 × warming
eCO2 × N
eCO2 × drought
Warming × N
Warming × drought
N × P
N × rainfall+
N × drought
Warming × rainfall+
Hedges' d++
Number of sample size
Additive
Antagonistic
Synergistic

Figure 5


(a)
eCO2 + warming
eCO2 + N addition
N addition + P addition
PN-C species
PN-C species
PN-P species
PN-C species
PN-P species
PN-C community
PN-P community
SN-C
SIN-C
Latitude
MAT
MAP
Magnitude
Duration
(b)
eCO2 × warming
eCO2 × N addition
N addition × P addition
PN-C species
PN-C species
PN-P species
PN-C species
PN-P species
PN-C community
PN-P community
Standardized total effects from SEM (unitless)
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00