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Soil pH-dependent nitrogen stimulation of plant biomass: magnesium and calcium as key constraints

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Summary

- Anthropogenic nitrogen (N) deposition can alleviate N limitation and stimulate plant growth in many terrestrial ecosystems. While theoretical models often emphasize phosphorus limitations as a constraint on this positive N effect, the impact of N-induced magnesium (Mg) and calcium (Ca) deficits due to soil acidification has been largely overlooked.
- Here, we synthesized data from 243 experiments across diverse terrestrial ecosystems to investigate the role of Mg and Ca in plant biomass responses to N addition.
- We found that the effect of N addition on aboveground biomass (AGB) shifted from neutral in low pH (≤ 4.5) to positive in medium (4.5–7.5) and high pH (> 7.5) soils. By contrast, belowground biomass (BGB) responses to N addition were independent of soil pH, leading to asymmetric increases in AGB and BGB. These variations in biomass accumulation across pH levels were primarily explained by changes in foliar Mg and Ca concentrations, which were negatively affected by N addition in low-pH soils but remained stable in medium and high-pH soils.
- Our findings underscore the critical role of Mg and Ca in modulating plant responses to N fertilization, providing new insights for improving Earth system models and better predicting climate–biosphere feedback.

Introduction

Nitrogen (N) is one of the most essential elements limiting terrestrial ecosystem productivity globally (Du *et al.*, 2020; Mason *et al.*, 2022). Anthropogenic N deposition has significantly altered the global N cycle (Lu *et al.*, 2018; Ackerman *et al.*, 2019), potentially alleviating N limitation in many ecosystems and promoting plant growth and biomass accumulation (LeBauer & Treseder, 2008; Xia & Wan, 2008; Thomas *et al.*, 2010; Fay *et al.*, 2015). However, the beneficial effects of N addition may be constrained by the low availability of other nutrients, as described by the 'law of the minimum' and the multiple resource limitation theory (Marschner, 2012; Fay *et al.*, 2015; Peñuelas *et al.*, 2019). Phosphorus (P) availability is widely recognized as a key constraint on N-stimulated biomass production, as N addition can aggravate P limitations (Y. Li *et al.*, 2016; Schulte-Uebbing & de Vries, 2018; Luo *et al.*, 2022). Despite frequent observations of N-induced soil magnesium (Mg) and calcium (Ca) depletion (Bowman *et al.*, 2008; Tian & Niu, 2015; Lu *et al.*, 2018), the influence of low Mg and Ca supply on plant biomass responses to N addition remains insufficiently understood.

Mg and Ca may play a more critical role than P in regulating plant biomass responses to N addition due to the distinct strategies plants use to acquire P vs base cations (Marschner, 2012). While plants can secrete phosphatases to access organic P sources

(Chen *et al.*, 2020; Luo *et al.*, 2022; Yu *et al.*, 2023), they lack comparable mechanisms for Mg and Ca uptake. Therefore, plant acquisition of these cations is largely dependent on their soil availability, which is strongly affected by soil pH. Base cations, including Mg and Ca, are integral in countering soil acidification (Fig. 1). During acidification, soil buffering mechanisms vary with pH: carbonates (e.g. $CaCO_3$) dominate when pH > 7.5, while base cations are the primary buffer in the pH range of 4.5–7.5 (Tian & Niu, 2015). When soil pH drops below 4.5, base cations become severely depleted, and aluminum (Al) and iron (Fe) cations are released from soils, resulting in Mg and Ca deficiencies while increasing Al and Fe concentrations (Bowman *et al.*, 2008). Such changes in soil cations can limit plant growth, cause branch dieback, and even lead to plant mortality, potentially offsetting or reversing the positive effects of N addition on biomass production (Magill *et al.*, 2004; Bowman *et al.*, 2008; Huang *et al.*, 2015). Conversely, N addition may enhance biomass production when Mg and Ca levels are adequate, which is more likely in soils with a higher pH (Feng *et al.*, 2019; Zhang *et al.*, 2021). Thus, the role of Mg and Ca in mediating plant responses to N addition may depend on soil pH, but this has not been well investigated to date.

Foliage and fine roots are among the most metabolically active plant tissues (Firn *et al.*, 2019; Bauters *et al.*, 2022). Changes in cation concentrations within these tissues can serve as indicators of plant cation status (Reich & Hinckley, 1980; Zhang *et al.*, 2021). This is particularly evident for Ca, which, once incorporated into plant cells, becomes immobile and cannot be reused (Yin *et al.*, 2022). Recent research highlights the importance of Mg and Ca for plant growth (Bauters *et al.*, 2022; Yin *et al.*, 2022), given their roles in key biochemical and physiological processes such as photosynthesis, respiration, ion balance regulation, and secondary metabolism (Rengel, 1992; Peñuelas *et al.*, 2019). Despite this, how their depletion following N enrichment affects plant productivity is still poorly understood. We hypothesize that plant biomass responses to N addition are largely mediated by soil pH, with variations driven by the presence of base cations in plant foliage and fine roots (Fig. 1).

To test the hypothesis, we conducted a meta-analysis of 243 global N addition field experiments (Supporting Information Fig. S1). We first assessed the overall effects of N addition on aboveground biomass (AGB), net primary productivity (ANPP), belowground biomass (BGB), total biomass (TB), and the fraction of BGB to TB (f_{BGB}) (Fig. 2). Using a random forest model, we identified soil pH as the most important predictor of plant biomass responses to N addition, alongside other variables such as N addition rates and durations, N forms, mean annual temperature (MAT), mean annual precipitation (MAP), latitude, and ecosystem types (Fig. S2). Based on these findings, we divided the dataset into three pH ranges (≤ 4.5, 4.5–7.5, and > 7.5) and compared biomass production responses to N addition across these ranges. To investigate the underlying mechanisms, we analyzed the effects of N addition on cation concentrations – including Mg, Ca, Al, Fe, and the Ca : Al ratio – in plant foliage, roots, and soils. These analyses were conducted for both the full dataset and within the different pH ranges. Collectively, our results provide new insights into how cation nutrients modulate the effects of N addition on plant biomass.

Materials and Methods

Data collection

In this study, we compiled the published data on plant biomass and cation elements in plant foliage, fine roots, and soils from N addition field experiments conducted world-wide. We expanded the dataset from our previous meta-analysis (Xu *et al.*, 2021) and the PlantNE database (Xu *et al.*, 2019) by searching the Web of Science, Google Scholar, and China National Knowledge Infrastructure (CNKI; http://www.cnki.net) to obtain plant biomass

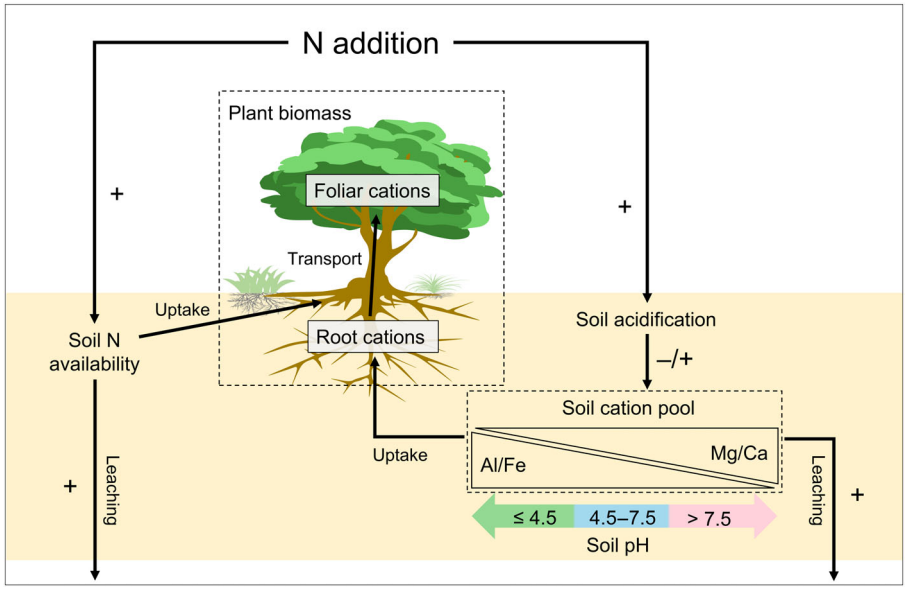

Fig. 1 A conceptual diagram illustrating the influence of nitrogen (N) addition on plant biomass accumulation through changes in the availability of soil N and nutrient cations in terrestrial ecosystems. Rectangles represent the pools of plant biomass or nutrient cations. The triangular arrow, transitioning from thick to thin along soil pH gradients (indicated by a colored dual-direction arrow: green, pH ≤ 4.5; blue, 4.5 < pH ≤ 7.5; pink, pH > 7.5), indicates a decrease in soil cation availability. The black arrow represents potential pathways through which N addition influences plant biomass or soil nutrients. The symbols '+' and '−/+' represent expected positive and unclear responses to N addition, respectively. Al, aluminum; Ca, calcium; Fe, iron; Mg, magnesium.

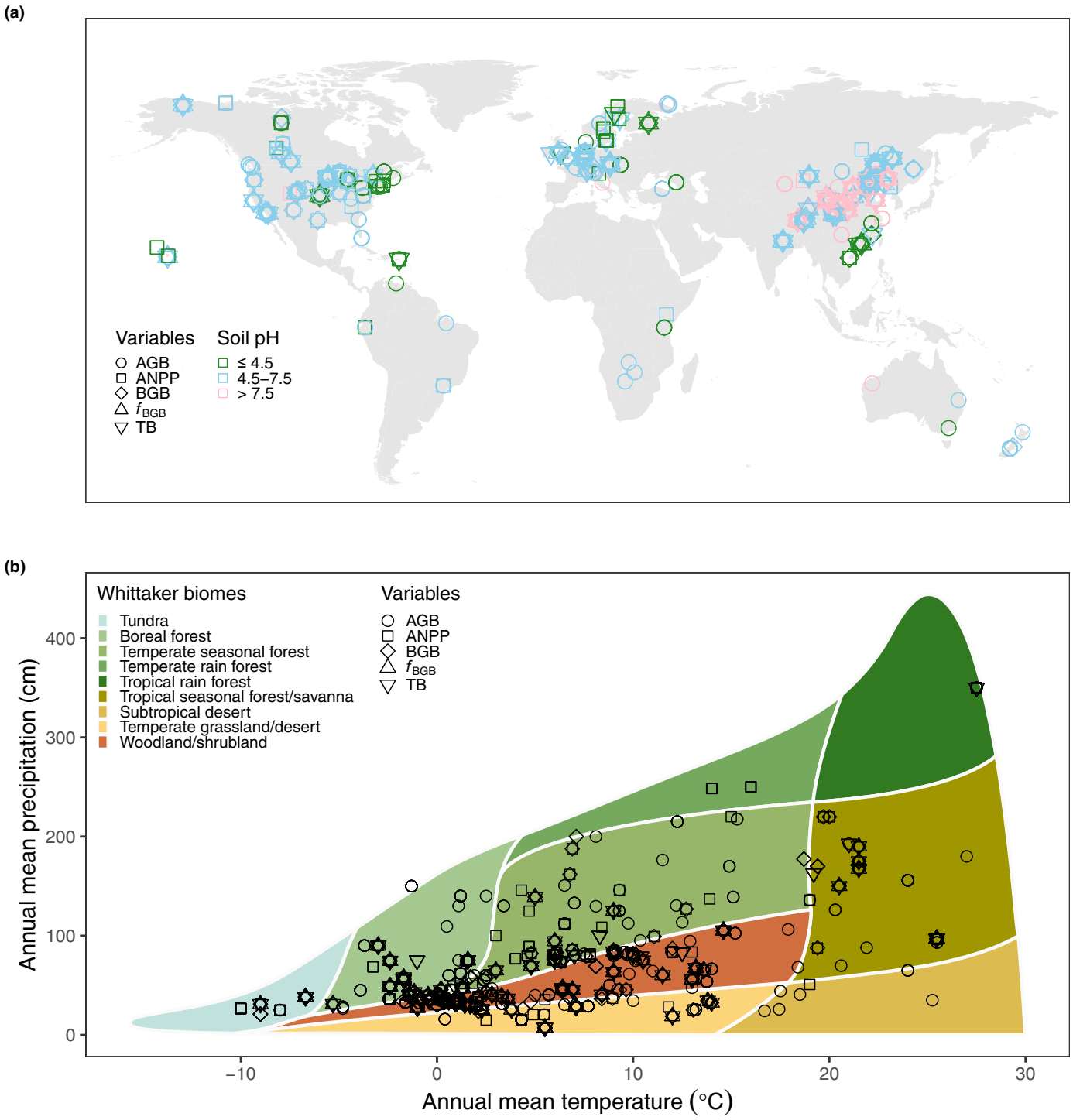

Fig. 2 Geographic and climatic distribution of experiments testing the effects of N addition on plant biomass collected for this meta-analysis. (a) The experimental sites were classified according to the measured variables and their corresponding soil pH levels. (b) The experimental sites were classified based on Whittaker biomass and the measured variables. AGB, aboveground biomass; ANPP, aboveground net primary productivity; BGB, belowground biomass; f_{BGB}, the fraction of belowground to total biomass; TB, total biomass.

data. We searched for peer-reviewed articles (up until February 2023) using the combination of terms "nitrogen addition or nitrogen deposition or nitrogen input or nitrogen fertilization or nitrogen enrichment or nitrogen supply" and "plant biomass or productivity or production or growth", not "crop or maize or wheat or rice". Studies were selected for biomass data collection based on the following criteria: (1) similar conditions between N treatment and control plots at the beginning of the field

experiment; (2) clearly described experimental methods and treatments, including the rates and durations of N addition; (3) a minimum experimental duration of 1 yr; (4) extractable means and sample sizes of variables; and (5) biomass production reported at the community or plot level. For studies with multiple experimental treatments, we only used data from the plots with N treatment and control. Observations from different N addition rates, forms of N, and ecosystem types within a single study were considered independent. When studies reported data for consecutive years, we only extracted data from the last year. For data presented graphically, we used GetData Graph Digitizer v.2.24 (http://getdata-graph-digitizer.com/) to extract relevant data from the graphs.

Within the biomass data, we observed biases in data for belowground net primary productivity (BNPP) across the ecosystems, with most BNPP data collected from grasslands and limited or no data from forests and other ecosystems. Therefore, we focused on the following variables: AGB and ANPP, BGB, TB, and the fraction of BGB to TB (f_{BGB}) to evaluate the effects of N addition on plant biomass production (Fig. 2). Our dataset included 1458 paired observations from 243 studies conducted in forests, grasslands, wetlands, shrublands, and tundra lands (Fig. S1). In addition to plant biomass, we also extracted data on experimental and environmental variables from publications. The N addition rate in our dataset ranged from 0.3 to 60 g N m^{-2} yr^{-1}, with experimental duration ranging from 1 to 58 yr. The forms of N used in experiments included inorganic N (including nitrate, ammonium, or both combined), organic N (urea), and mixed N (the combination of inorganic and organic N), as described in a previous study (Rocci *et al.*, 2021). We derived MAT and MAP data from the WorldClim database (https://www.worldclim.org) when the climate data were not reported in the publications. The MAT values ranged from −10°C to 27.5°C, and the MAP values ranged from 72 to 3500 mm. Soil pH data were obtained from publications or the SoilGrids database (https://www.isric.org/explore/soilgrids) when not reported, with soil pH ranging from 3.20 to 9.98. We also recorded the longitude and latitude of each experimental location from the publications.

To obtain data on cation elements such as Mg, Ca, Fe, Al, and Ca : Al ratio in foliage, fine roots, and soils, we first extracted these variables from a subset of publications. Because few studies simultaneously reported data on plant biomass and cation elements in foliage, fine roots, and soils, we searched for additional peer-reviewed articles up to February 2023. The search terms included "nitrogen addition or nitrogen deposition or nitrogen input or nitrogen fertilization or nitrogen enrichment or nitrogen supply", "foliar or leaf or fine root or root or soil", and "Ca or Mg or Al or Fe or stoichiometry". The literature selection criteria followed the same procedure used for biomass data. We also supplemented our database with data from two previous meta-analyses (Tian & Niu, 2015; Mao *et al.*, 2020). In total, we collected 1493 data points on cations from 83 studies.

Meta-analysis

We used the log response ratio (lnRR) (Hedges *et al.*, 1999) to estimate the effect size of N addition on plant biomass and cationic elements as follows: $\ln RR = \ln(X_t/X_c)$, where X_t and X_c are the mean values of the biomass or cationic variable within the N addition and control treatments from each study, respectively. Since sampling variances were not reported for 22% of the individual observations in our dataset, we calculated the weighting factors (Wn) using replications (Pittelkow *et al.*, 2015; Mao *et al.*, 2020) as $Wn = (n_t \times n_c)/(n_t + n_c)$, where n_t and n_c are the number of replicates under N addition and control treatments, respectively. For foliar cations, more than half of the studies reported data at the species level, so we adjusted the weights of observations from these studies by the total number of species (n_S) when multiple species were included in an experiment (Mao *et al.*, 2020). The final weight (Wn′) for foliar cation was calculated as $Wn' = Wn/n_S$.

We analyzed the effect size of N addition using a weighted mixed-effect model with 'Study' as a random effect, using the lme4 package in R (Bates *et al.*, 2015). The random effect accounted for possible autocorrelation among observations within each study. Since BGB was measured from several soil layers, we also added 'Soil layer' as a random effect to account for soil layer effects when estimating the effect sizes of N addition on BGB. The models were fitted using a restricted maximum likelihood algorithm, and a bootstrap procedure with 999 iterations was applied to generate the overall effect and associated 95% confidence intervals (CIs). When the 95% CIs did not overlap with zero, we considered the effect of N addition on the selected variable to be significant. For ease of interpretation in the figures and main text, we transformed the effects of N addition on biomass and cations into percentage.

To determine the potential drivers influencing the response of plant biomass to N addition, we coded eight variables (e.g. the rate and duration of N addition, N form, ecosystem types, MAT, MAP, latitude, and soil pH). Due to data limitations, we excluded variables related to the response of cation elements to N addition. We used a random forest model to identify the most important variables predicting the response of plant biomass to N addition for the complete dataset. The random forest modeling approach can handle complex relationships between multiple predictor variables and address overfitting (Breiman, 2001; Strobl *et al.*, 2008). The model was trained to improve the predictive performance of the variables using 10-fold cross-validation, with 75% of the data used for training and the remaining 25% for validation. Model training was performed using the 'train' function from the caret package in R (Kuhn, 2008), and the relative importance of variables was ranked by mean decrease in node impurity (Liaw & Wiener, 2002). The results showed that soil pH was the most important variable influencing the effects of N addition on plant biomass production (Fig. S2).

We conducted a subgroup analysis to analyze differences in plant biomass response to N addition across different soil pH ranges using a mixed model with 'Study' as a random effect. Before analysis, we divided the soil pH into three groups (≤ 4.5, 4.5–7.5, and > 7.5), based on different buffering mechanisms against soil acidification as described by Bowman *et al.* (2008) and Tian & Niu (2015). We also tested whether the pH pattern of biomass responses is generally applied to different N addition

rates, durations, forms, and ecosystem types using a mixed model with 'Study' as a random effect. N addition rates and durations were portioned into divergent groups based on Song *et al.* (2019) and Rocci *et al.* (2021). Due to limited data availability, this analysis was restricted to forest, grassland, and wetland ecosystems.

We conducted a sensitivity analysis to ensure the robustness of our findings. First, we calculated the weighting factor of each observation using the inverse of the pooled variance (v):

$$v = \frac{S_t^2}{n_t \overline{X}_t^2} + \frac{S_c^2}{n_c \overline{X}_c^2}$$

where S_t and S_c are the SD, n_t and n_c are the replicates, and X_t and X_c are the means for the variables under N addition and control treatments, respectively. Due to data limitations, this analysis was restricted to the subset of studies that reported sampling variance. The effect size and its 95% CI were calculated in a weighted mixed-effect model with 'Study' as a random effect, using the METAFOR package (Viechtbauer, 2010). Second, we incorporated 'Ecosystem' as a random effect in the mixed model to account for potential ecosystem-specific effects. Third, we performed a leave-one-out analysis to assess whether any single study disproportionately influenced the results. These analyses collectively confirmed the reliability and generalizability of our results (Figs S3–S5).

The responses of foliar and root cationic elements to N addition across soil pH groups were analyzed using a mixed model with 'Study' as a random effect. We further tested the relationships between plant biomass and foliar cation elements' responses to N addition based on paired data.

Finally, we assessed the potential for publication bias using Egger's test for funnel plot asymmetry on mixed-effect models. Results showed no significant publication bias in our meta-analysis (Table S1).

Results and Discussion

N addition increased plant biomass world-wide

N addition significantly increased plant AGB by 40.4% (95% CI: ±7.8%; $P < 0.001$; Fig. 3a), ANPP by 27.2% (95% CI: ±10.2%; $P < 0.001$; Fig. S6a), BGB by 19.2% (95% CI: ±10.2%; $P < 0.001$; Fig. 4a), and TB by 23.5% (95% CI: ±9.2%; $P < 0.001$; Fig. S7a) across all experiments. These results strongly support the idea that plant growth in terrestrial ecosystems is generally N-limited, consistent with previous studies (LeBauer & Treseder, 2008; Xia & Wan, 2008; Thomas *et al.*, 2010). Additionally, N addition decreased the f_{BGB} by an average of 6.5% (95% CI: ±3.5%; $P < 0.001$; Fig. S8a), suggesting that plants allocate fewer resources to belowground structures under N-enriched conditions (Song *et al.*, 2019; Feng *et al.*, 2023). This shift likely enhances light interception and photosynthesis when more resources, including N, are available (Janssens *et al.*, 2010; Li *et al.*, 2020; Feng *et al.*, 2023). Given that the contribution to long-term C sequestration from belowground inputs is generally more efficient than from aboveground inputs (Jackson *et al.*, 2017; Sokol & Bradford, 2019), our findings suggest that N addition may have significant implications for the soil C cycle by reducing f_{BGB}.

Soil pH-dependent responses of biomass to N addition

The effects of N addition on AGB ($P < 0.001$; Fig. 3a) and ANPP ($P < 0.001$; Fig. S6a) were positively correlated with soil pH. When partitioning biomass data into three soil pH ranges, we found that N addition significantly increased AGB and ANPP at pH > 4.5 but had no significant impact at pH ≤ 4.5 (Figs 3b, S6b). By contrast, BGB responses to N addition did not vary significantly across different pH levels (Fig. 4a,b; Table S2). These patterns were consistently observed across different N addition rates and durations, ecosystem types, and N forms (Figs 3c, 4c, S6c; Table S2), indicating that the influence of soil pH on plant biomass responses is globally consistent – AGB increases with higher soil pH, while BGB remains stable. Furthermore, the response of TB to N addition mirrored that of AGB (Figs 3, S7), and f_{BGB} exhibited nonsignificant changes or a reduction in response to N addition at both pH ≤ 4.5 and > 4.5 (Fig. S8b; Table S2), suggesting that AGB plays a directive role in biomass accumulation and allocation.

As hypothesized, the pH-dependent responses in AGB were linked to N-induced changes in foliar cation concentrations. At pH ≤ 4.5, N addition did not affect Al and Fe, but it reduced Mg, Ca, and the Ca : Al ratio in the foliage (Fig. 5a). Similar responses were observed in soil concentrations of these cations (Fig. S9), providing robust evidence that N addition induced Mg and Ca deficiency and potentially elevated Al stress (Rengel, 1992; Mao *et al.*, 2020). Given the essential roles of Mg and Ca in plant growth (Peñuelas *et al.*, 2019; Tian *et al.*, 2021; Bauters *et al.*, 2022), their deficiency likely restricts biomass increments, eliminating N-stimulated AGB (Sikström, 1997; Battles *et al.*, 2014; Z. Li *et al.*, 2016). Additionally, increased Al stress may limit plant growth, as Al is toxic to plants (Magill *et al.*, 2004; Bowman *et al.*, 2008; Huang *et al.*, 2015), and reduced Ca : Al ratios could further hinder AGB responses to N addition (Magill *et al.*, 2004). At pH > 4.5, although soil exchangeable Mg and Ca concentrations decreased and Al concentrations increased (Fig. S9), N addition did not cause Mg or Ca deficiencies or increase Al stress to plants (Fig. 5). This allowed for the stimulation of AGB and ANPP by N (Figs 3, S6). Although foliar Fe decreased with N addition at a pH range of 4.5–7.5 ($P < 0.05$; Fig. 5a), this reduction did not disrupt N-stimulated AGB, as observed in previous studies (Cai *et al.*, 2017; Feng *et al.*, 2019). These findings suggest that divergent AGB responses along soil pH gradients are at least partly related to shifts in the effects of N addition on foliar Mg and Ca, from negative at pH ≤ 4.5 to neutral at pH > 4.5.

The consistent positive BGB response to N addition across all pH ranges (Fig. 4b) may reflect increased plant uptake of Mg and Ca, as evidenced by unchanged root concentrations of these cations (Fig. 5b). Additionally, increased Mg and Ca uptake may be coupled with heightened transpiration (Gilliham *et al.*, 2011;

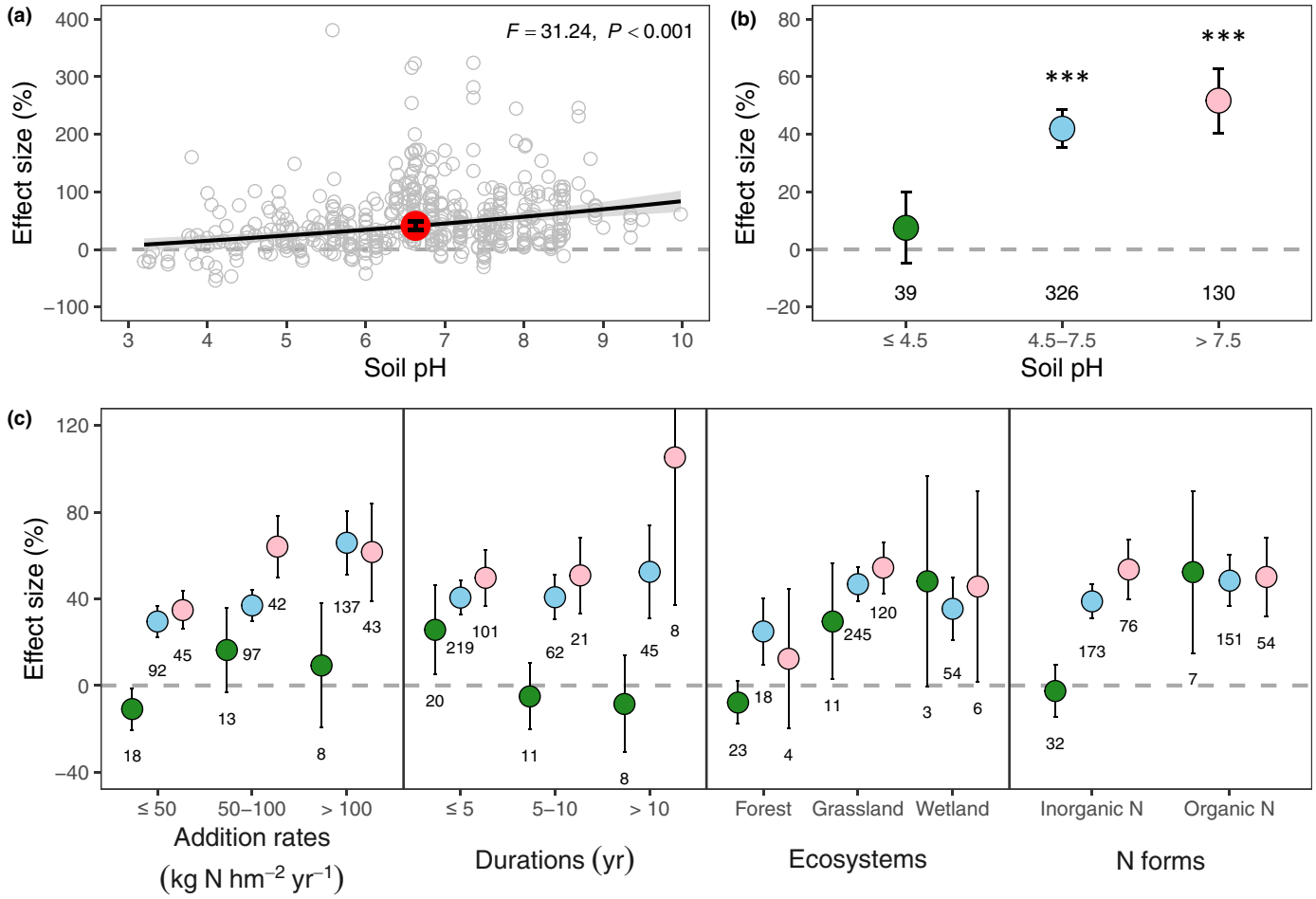

Fig. 3 Effect sizes of nitrogen (N) addition on plant aboveground biomass (AGB). (a) Variations in effect sizes as a function of soil pH. Scattered dots represent the original effect size of N addition, with the overall mean and 95% confidence intervals (CI) shown as a red point and associated error bar. (b) The effect sizes of N addition on AGB across different soil pH ranges. Statistical significance was determined using a Student's *t*-test: ***, $P < 0.001$. (c) Comparison of AGB responses across soil pH ranges within different study categories, including N addition rates and durations, ecosystem types, and N forms, with color use following the scheme in (b). The effect of N addition was considered significant when the 95% CI of the effect size did not overlap with zero. Colored points and associated error bars in (b, c) represent the mean ± 95% CI of the effects of N addition, with the number of observations indicated below each point.

Green *et al.*, 2013), triggering water demand, as these cations are passively acquired (Lu *et al.*, 2018; Yin *et al.*, 2022). Therefore, plants likely allocate additional resources to roots to meet increased water uptake demands (Peng & Yang, 2016; Yue *et al.*, 2021), causing BGB to increase under N enrichment across all pH ranges.

We also considered the role of other essential nutrients, such as P and potassium (K) (Fay *et al.*, 2015; Y. Li *et al.*, 2016; Schulte-Uebbing & de Vries, 2018), in plant biomass responses to N addition across soil pH ranges. Given that P and K availability is generally low in strongly acidic soils (Bauters *et al.*, 2022), the neutral effects of N addition on AGB may be driven by N-induced P or K limitation. However, our results showed that N addition did not significantly decrease P concentrations in foliage, fine roots, or soils at pH ≤ 4.5 (Fig. S10), consistent with findings from previous studies (Högberg *et al.*, 2006; Lu *et al.*, 2018; Yu *et al.*, 2023). Similarly, although soil exchangeable K decreased, N addition had no detectable impact on K concentrations in foliage or fine roots at pH ≤ 4.5 (Fig. S10). These findings, along with the absence of significant relationships between AGB responses and foliar P and K concentrations (Fig. S11), suggest that P and K availability alone cannot fully account for the varying AGB responses to N addition. To further explore potential mechanisms, we investigated whether the neutral effects of N addition were driven by reduced foliar N. Contrary to this hypothesis, N addition increased N concentrations in both foliage and fine roots at pH ≤ 4.5 (Fig. S12), suggesting that the lack of an AGB response is not due to insufficient N uptake. Instead, our analysis highlights the critical roles of Mg and Ca cations in modulating biomass responses to N addition, further illustrated by the positive correlations between AGB and foliar Ca, Mg, and the Ca : Al ratio, as well as between foliar Ca and Mg under N addition (Fig. 6). Thus, variations in plant Mg and Ca status likely contribute to the shifting

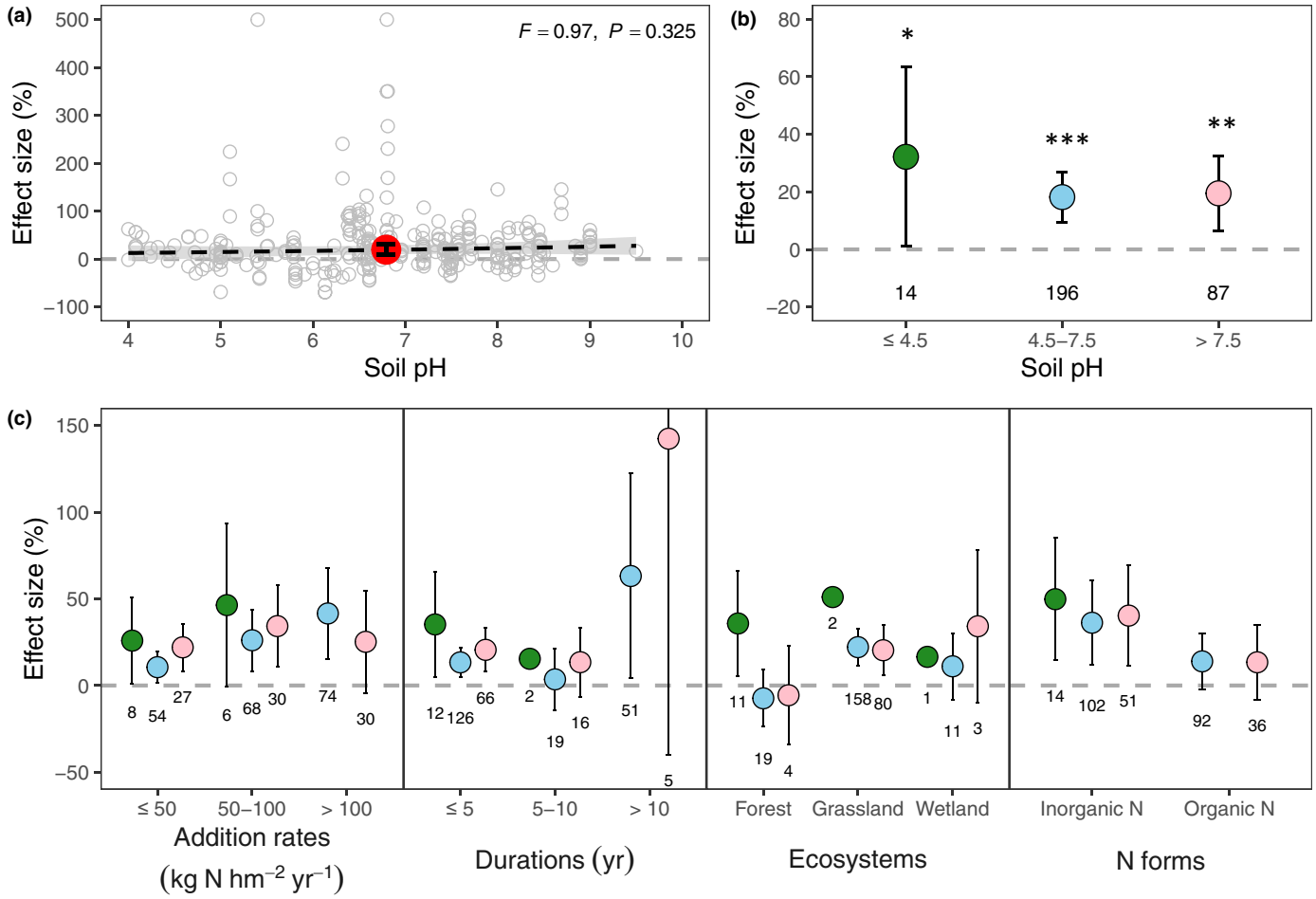

Fig. 4 Effect sizes of nitrogen (N) addition on plant belowground biomass (BGB). (a) Variations in effect sizes as a function of soil pH. Scattered dots represent the original effect size of N addition, with the overall mean and 95% confidence intervals (CI) shown as a red point and associated error bar. (b) The effect sizes of N addition on BGB across different soil pH ranges. Statistical significance was determined using a *t*-test, with *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (c) Comparison of BGB responses across different soil pH ranges within various study categories, including N addition rates and durations, ecosystem types, and N forms, with color usage consistent with (b). The effect of N addition was considered significant when the 95% CI of the effect size did not overlap with zero. Colored points and associated error bars in (b, c) represent the mean ± 95% CI of the effects of N addition, with the number of observations indicated below each point.

biomass responses to N addition across different soil pH ranges. Future research linking biomass dynamics with plant nutrient status is needed to enhance our understanding of how cation nutrients, particularly Mg and Ca, influence biomass responses under N addition.

Implications

Our findings have significant implications for predicting biomass accumulation under N deposition. First, we identified a pH-dependent response in AGB, ANPP, and TB to N addition, with no change observed at soil pH ≤ 4.5 and significant increases at soil pH > 4.5. The pH-dependent response will significantly improve the prediction of plant growth and C sequestration in global terrestrial ecosystems under N deposition, especially in temperate regions generally classified as N-limited (Schulte-Uebbing & de Vries, 2018; Du *et al.*, 2020). Given that soil pH in some temperate regions is low (≤ 4.5) (Magill *et al.*, 2004; Bowman *et al.*, 2008), our findings suggest that N addition does not uniformly stimulate biomass accumulation across temperate ecosystems. Therefore, estimates of biomass accumulation driven by N deposition in temperate ecosystems could be underestimated in areas with pH > 4.5 and overestimated in those with pH ≤ 4.5 if pH-dependent responses are not considered.

Second, recognizing pH-dependent variability in plant biomass provides an insight into the inconsistent effects of N addition on AGB and ANPP across ecosystem types and MAP gradients (LeBauer & Treseder, 2008; Xia & Wan, 2008; Yan *et al.*, 2019). For instance, grasslands, which tend to have higher soil pH than forests, exhibited a stronger AGB and ANPP response to N addition (Fig. S13). Similarly, in regions where water is not limiting (MAP > 200–300 mm), N addition had a greater effect on AGB and ANPP in drier climates than in humid

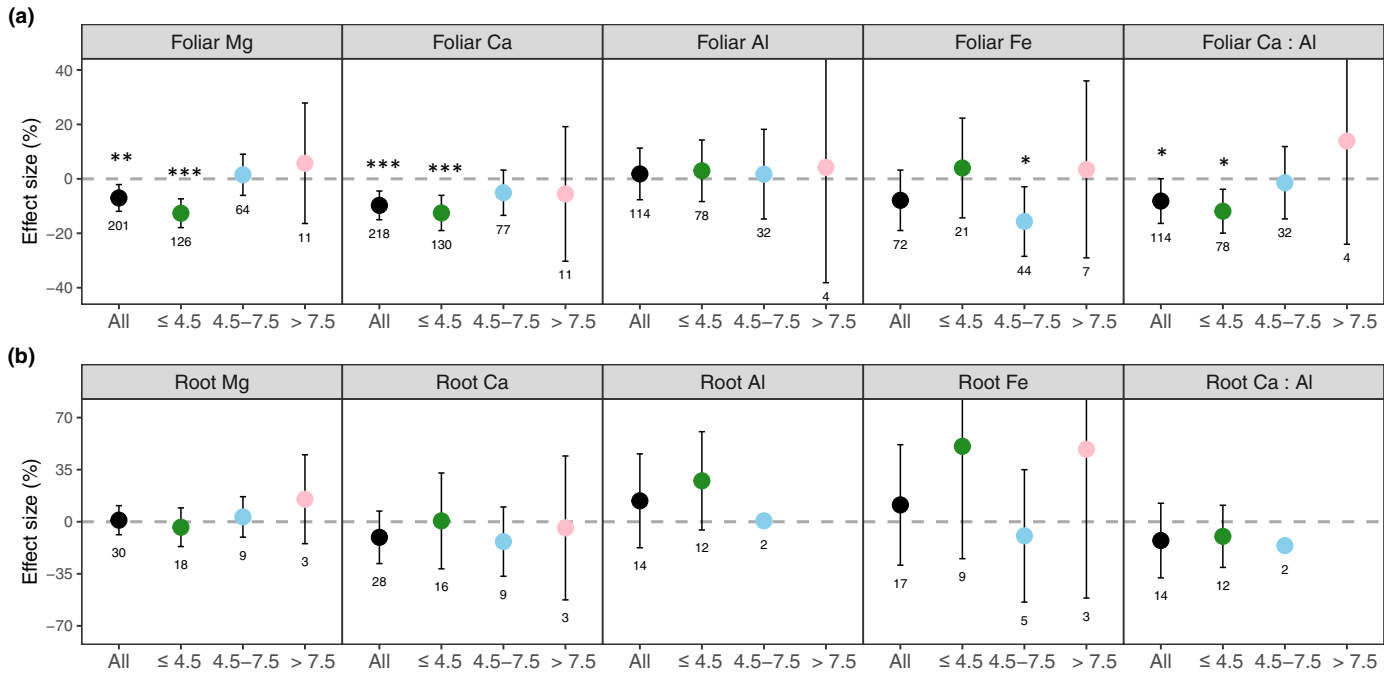

Fig. 5 Effects of nitrogen (N) addition on cation elements and their ratios in (a) plant foliage and (b) fine roots across the entire dataset and within different soil pH ranges. Black and colored points, along with associated error bars, represent the mean ± 95% confidence intervals measuring the effects of N addition, with the number of observations indicated below each point. Statistical significance is determined using a Student's *t*-test: with *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Al, aluminum; Ca, calcium; Ca : Al, calcium to aluminum ratio; Fe, iron; Mg, magnesium.

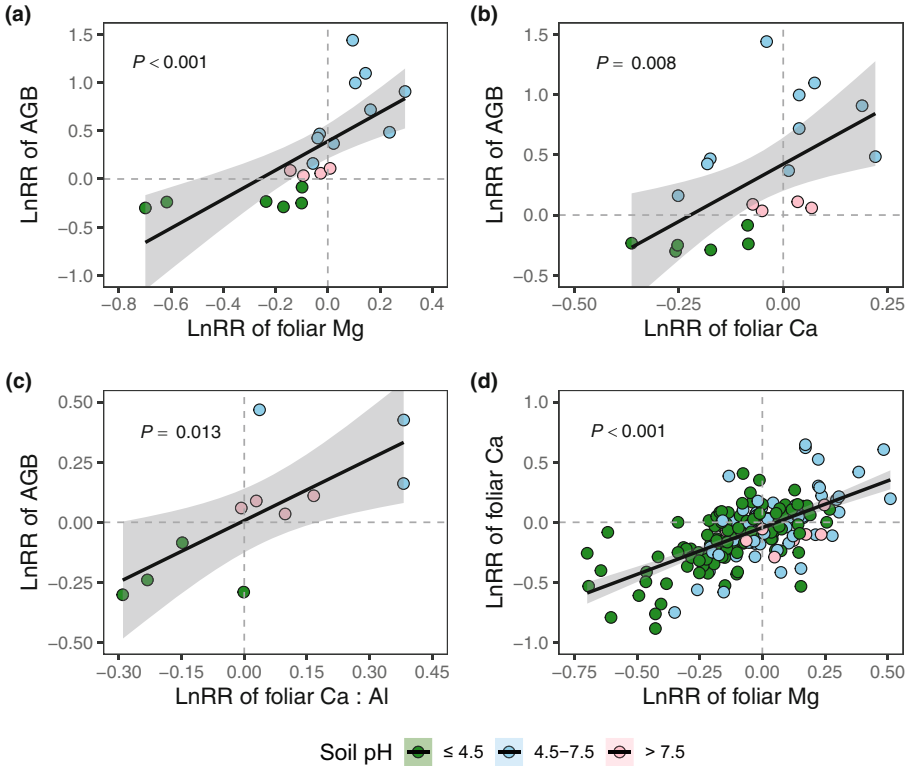

Fig. 6 Relationship between aboveground biomass (AGB) and foliar magnesium (Mg; a), calcium (Ca; b), calcium-to-aluminum ratio (Ca : Al; c), and foliar Ca and Mg (d) under N enrichment conditions. The shaded areas represent the 95% confidence intervals of regression lines. An *F*-test was used to calculate *P* values.

regions, aligning with the trend of decreasing soil pH as MAP increases ($P < 0.05$; Fig. S14). Thus, incorporating pH-dependent AGB responses into Earth system models is crucial for improving predictions of carbon dynamics under future N deposition scenarios.

Third, our results provide evidence that shifts in plant biomass responses to N addition are strongly linked to N-induced changes in foliar Mg and Ca concentrations across soil pH gradients. At soil pH ≤ 4.5, Mg and Ca deficiencies, as indicated by reduced concentrations of these cations in both foliage and soils, diminish the stimulatory effect of N on AGB while enhancing BGB, resulting in neutral TB responses. By contrast, at pH > 4.5, the absence of Mg and Ca deficiencies enables N addition to increase both AGB and BGB. These findings highlight the critical role of Mg and Ca cations in plant responses to N fertilization, providing new mechanistic insights for improving Earth system models that predict climate–biosphere feedbacks.

Conclusions

As N inputs into terrestrial ecosystems continue to rise due to fertilization and atmospheric deposition (Ackerman *et al.*, 2019), understanding the mechanisms driving plant biomass production is critical for predicting future impacts on the carbon cycle (Lu *et al.*, 2018; Song *et al.*, 2019). This meta-analysis highlights the global role of N addition in promoting plant biomass, particularly through enhanced aboveground allocation. Our study addresses inconsistencies in prior research, revealing that N addition's effects on AGB and TB are strongly influenced by soil pH, shifting from neutral at pH ≤ 4.5 to positive at pH > 4.5, while BGB responses remain independent of pH. These findings indicate that N addition does not always lead to increased AGB and TB, especially in highly acidic soils where N-induced reductions in Mg and Ca can pose significant threats to ecosystem productivity and health.

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Competing interests

None declared.

Author contributions

All the authors contributed intellectual content, rendered research assistance, and/or involved in manuscript preparation. XX and CX conceived the idea and designed the study. CX collected and analyzed the data and visualized the results with contributions from XX, PR, HYHC, YL and WF. CX and XX wrote the manuscript with input from JP, JS, PR, HYHC, YL, XZ, WF, CJ, ML, JC, WL, XC and JW.

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Data availability

The data and code supporting the findings of this study are available at doi: 10.6084/m9.figshare.27322995.v1.

References

Ackerman D, Millet DB, Chen X. 2019. Global estimates of inorganic nitrogen deposition across four decades. *Global Biogeochemical Cycles* **33**: 100–107.

Bates D, Mächler M, Bolker B, Walker S. 2015. Fitting linear mixed-effects models using LME4. *Journal of Statistical Software* **67**: 201–210.

Battles JJ, Fahey TJ, Driscoll CT, Blum JD, Johnson CE. 2014. Restoring soil calcium reverses forest decline. *Environmental Science and Technology Letters* **1**: 15–19.

Bauters M, Janssens IA, Wasner D, Doetterl S, Vermeir P, Griepentrog M, Drake TW, Six J, Barthel M, Baumgartner S *et al.* 2022. Increasing calcium scarcity along Afrotropical forest succession. *Nature Ecology & Evolution* **6**: 1122–1131.

Bowman WD, Cleveland CC, Halada Ľ, Hreško J, Baron JS. 2008. Negative impact of nitrogen deposition on soil buffering capacity. *Nature Geoscience* **1**: 767–770.

Breiman L. 2001. Random forests. *Machine Learning* **45**: 5–32.

Cai J, Weiner J, Wang R, Luo W, Zhang Y, Liu H, Xu Z, Li H, Zhang Y, Jiang Y. 2017. Effects of nitrogen and water addition on trace element stoichiometry in five grassland species. *Journal of Plant Research* **130**: 659–668.

Chen J, van Groenigen KJ, Hungate BA, Terrer C, van Groenigen JW, Maestre FT, Ying SC, Luo Y, Jørgensen U, Sinsabaugh RL *et al.* 2020. Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems. *Global Change Biology* **26**: 5077–5086.

Du E, Terrer C, Pellegrini AFA, Ahlström A, van Lissa CJ, Zhao X, Xia N, Wu X, Jackson RB. 2020. Global patterns of terrestrial nitrogen and phosphorus limitation. *Nature Geoscience* **13**: 221–226.

Fay PA, Prober SM, Harpole WS, Knops JMH, Bakker JD, Borer ET, Lind EM, MacDougall AS, Seabloom EW, Wragg PD *et al.* 2015. Grassland productivity limited by multiple nutrients. *Nature Plants* **1**: 15080.

Feng H, Guo J, Peng C, Kneeshaw D, Roberge G, Pan C, Ma X, Zhou D, Wang W. 2023. Nitrogen addition promotes terrestrial plants to allocate more biomass to aboveground organs: a global meta-analysis. *Global Change Biology* **29**: 3970–3989.

Feng X, Wang R, Yu Q, Cao Y, Zhang Y, Yang L, Dijkstra FA, Jiang Y. 2019. Decoupling of plant and soil metal nutrients as affected by nitrogen addition in a meadow steppe. *Plant and Soil* **443**: 337–351.

Firn J, McGree JM, Harvey E, Flores-Moreno H, Schütz M, Buckley YM, Borer ET, Seabloom EW, La Pierre KJ, MacDougall AM *et al.* 2019. Leaf nutrients, not specific leaf area, are consistent indicators of elevated nutrient inputs. *Nature Ecology & Evolution* **3**: 400–406.

Gilliham M, Dayod M, Hocking BJ, Xu B, Conn SJ, Kaiser BN, Leigh RA, Tyerman SD. 2011. Calcium delivery and storage in plant leaves: exploring the link with water flow. *Journal of Experimental Botany* **62**: 2233–2250.

Green MB, Bailey AS, Bailey SW, Battles JJ, Campbell JL, Driscoll CT, Fahey TJ, Lepine LC, Likens GE, Ollinger SV *et al.* 2013. Decreased water flowing from a forest amended with calcium silicate. *Proceedings of the National Academy of Sciences, USA* **110**: 5999–6003.

Hedges LV, Gurevitch J, Curtis PS. 1999. The meta-analysis of response ratios in experimental ecology. *Ecology* **80**: 1150–1156.

Högberg P, Fan H, Quist M, Binkley D, Tamm CO. 2006. Tree growth and soil acidification in response to 30 years of experimental nitrogen loading on boreal forest. *Global Change Biology* **12**: 489–499.

Huang Y, Kang R, Mulder J, Zhang T, Duan L. 2015. Nitrogen saturation, soil acidification, and ecological effects in a subtropical pine forest on acid soil in southwest China. *Journal of Geophysical Research: Biogeosciences* **120**: 2457–2472.

Jackson RB, Lajtha K, Crow SE, Hugelius G, Kramer MG, Piñeiro G. 2017. The ecology of soil carbon: pools, vulnerabilities, and biotic and abiotic controls. *Annual Review of Ecology, Evolution, and Systematics* **48**: 419–445.

Janssens IA, Dieleman W, Luyssaert S, Subke J-A, Reichstein M, Ceulemans R, Ciais P, Dolman AJ, Grace J, Matteucci G *et al.* 2010. Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience* **3**: 315–322.

Kuhn M. 2008. Building predictive models in R using the caret package. *Journal of Statistical Software* **28**: 1–26.

LeBauer DS, Treseder KK. 2008. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* **89**: 371–379.

Li W, Zhang H, Huang G, Liu R, Wu H, Zhao C, McDowell NG. 2020. Effects of nitrogen enrichment on tree carbon allocation: a global synthesis. *Global Ecology and Biogeography* **29**: 573–589.

Li Y, Niu S, Yu G. 2016. Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Global Change Biology* **22**: 934–943.

Li Z, Dai P, Wang Y, Li T, Webb AA, Wang Y, Li Z, Kou T, Shi G, Zhang B. 2016. Effects of liming on health and growth of young *Schima superba* trees under canopy of a *Pinus massoniana* stand damaged by soil acidification in Chongqing, China. *New Forests* **47**: 801–813.

Liaw A, Wiener M. 2002. Classification and regression by randomForest. *R News* **2**: 18–22.

Lu X, Vitousek PM, Mao Q, Gilliam FS, Luo Y, Zhou G, Zou X, Bai E, Scanlon TM, Hou E *et al.* 2018. Plant acclimation to long-term high nitrogen deposition in an N-rich tropical forest. *Proceedings of the National Academy of Sciences, USA* **115**: 5187–5192.

Luo M, Moorhead DL, Ochoa-Hueso R, Mueller CW, Ying SC, Chen J. 2022. Nitrogen loading enhances phosphorus limitation in terrestrial ecosystems with implications for soil carbon cycling. *Functional Ecology* **36**: 2845–2858.

Magill AH, Aber JD, Currie WS, Nadelhoffer KJ, Martin ME, McDowell WH, Melillo JM, Steudler P. 2004. Ecosystem response to 15 years of chronic nitrogen additions at the Harvard Forest LTER, Massachusetts, USA. *Forest Ecology and Management* **196**: 7–28.

Mao J, Mao Q, Zheng M, Mo J. 2020. Responses of foliar nutrient status and stoichiometry to nitrogen addition in different ecosystems: a meta-analysis. *Journal of Geophysical Research: Biogeosciences* **125**: 1–16.

Marschner H. 2012. *Marschner's mineral nutrition of higher plants*. Amsterdam, the Netherlands: Elsevier.

Mason RE, Craine JM, Lany NK, Jonard M, Ollinger SV, Groffman PM, Fulweiler RW, Angerer J, Read QD, Reich PB *et al.* 2022. Evidence, causes, and consequences of declining nitrogen availability in terrestrial ecosystems. *Science* **376**: eabh3767.

Peng Y, Yang Y. 2016. Allometric biomass partitioning under nitrogen enrichment: evidence from manipulative experiments around the world. *Scientific Reports* **6**: 28918.

Peñuelas J, Fernández-Martínez M, Ciais P, Jou D, Piao S, Obersteiner M, Vicca S, Janssens IA, Sardans J. 2019. The bioelements, the elementome, and the biogeochemical niche. *Ecology* **100**: 1–15.

Pittelkow CM, Liang X, Linquist BA, Van Groenigen LJ, Lee J, Lundy ME, Van Gestel N, Six J, Venterea RT, Van Kessel C. 2015. Productivity limits and potentials of the principles of conservation agriculture. *Nature* **517**: 365–368.

Reich PB, Hinckley TM. 1980. Water relations: soil fertility, and plant nutrient composition of a pygmy oak ecosystem. *Ecology* **61**: 400–416.

Rengel Z. 1992. Role of calcium in aluminium toxicity. *New Phytologist* **121**: 499–513.

Rocci KS, Lavallee JM, Stewart CE, Cotrufo MF. 2021. Soil organic carbon response to global environmental change depends on its distribution between mineral-associated and particulate organic matter: a meta-analysis. *Science of the Total Environment* **793**: 148569.

Schulte-Uebbing L, de Vries W. 2018. Global-scale impacts of nitrogen deposition on tree carbon sequestration in tropical, temperate, and boreal forests: a meta-analysis. *Global Change Biology* **24**: e416–e431.

Sikström U. 1997. Effects of low-dose liming and nitrogen fertilization on stemwood growth and needle properties of *Picea abies* and *Pinus sylvestris*. *Forest Ecology and Management* **95**: 261–274.

Sokol NW, Bradford MA. 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. *Nature Geoscience* **12**: 46–53.

Song J, Wan S, Piao S, Knapp AK, Classen AT, Vicca S, Ciais P, Hovenden MJ, Leuzinger S, Beier C *et al.* 2019. A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. *Nature Ecology & Evolution* **3**: 1309–1320.

Strobl C, Boulesteix A-L, Kneib T, Augustin T, Zeileis A. 2008. Conditional variable importance for random forests. *BMC Bioinformatics* **9**: 307.

Thomas RQ, Canham CD, Weathers KC, Goodale CL. 2010. Increased tree carbon storage in response to nitrogen deposition in the US. *Nature Geoscience* **3**: 13–17.

Tian D, Niu S. 2015. A global analysis of soil acidification caused by nitrogen addition. *Environmental Research Letters* **10**: 024019.

Tian XY, He DD, Bai S, Zeng WZ, Wang Z, Wang M, Wu LQ, Chen ZC. 2021. Physiological and molecular advances in magnesium nutrition of plants. *Plant and Soil* **468**: 1–17.

Viechtbauer W. 2010. Conducting meta-analyses in R with the METAFOR package. *Journal of Statistical Software* **36**: 1–48.

Xia J, Wan S. 2008. Global response patterns of terrestrial plant species to nitrogen addition. *New Phytologist* **179**: 428–439.

Xu C, Xu X, Ju C, Chen HYH, Wilsey BJ, Luo Y, Fan W. 2021. Long-term, amplified responses of soil organic carbon to nitrogen addition worldwide. *Global Change Biology* **27**: 1170–1180.

Xu X, Lu R, Yan L, Xia J. 2019. PlantNE: a global database of plant biomass from nitrogen-addition experiments. *Ecology* **100**: e02840.

Yan L, Xu X, Xia J. 2019. Different impacts of external ammonium and nitrate addition on plant growth in terrestrial ecosystems: a meta-analysis. *Science of the Total Environment* **686**: 1010–1018.

Yin Y, Zhou Y-B, Li H, Zhang S-Z, Fang Y, Zhang Y-J, Zou X. 2022. Linking tree water use efficiency with calcium and precipitation. *Tree Physiology* **42**: 2419–2431.

Yu G, Chen J, Yu M, Li A, Wang Y, He X, Tang X, Liu H, Jiang J, Mo J *et al.* 2023. Eighteen-year nitrogen addition does not increase plant phosphorus demand in a nitrogen-saturated tropical forest. *Journal of Ecology* **111**: 1545–1560.

Yue K, Fornara DA, Li W, Ni X, Peng Y, Liao S, Tan S, Wang D, Wu F, Yang Y. 2021. Nitrogen addition affects plant biomass allocation but not allometric relationships among different organs across the globe. *Journal of Plant Ecology* **14**: 361–371.

Zhang J, Ren T, Yang J, Xu L, Li M, Zhang Y, Han X, He N. 2021. Leaf multi-element network reveals the change of species dominance under nitrogen deposition. *Frontiers in Plant Science* **12**: 1–11.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Fig. S1 PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) diagram illustrating the process of filtering studies on plant biomass that were included in this meta-analysis.

Fig. S2 Relative importance of selected variables in predicting plant biomass responses to N addition.

Fig. S3 Effects of N addition on plant biomass across different soil pH levels were analyzed using studies that reported sampling variance.

Fig. S4 Effects of N addition on plant biomass across different soil pH levels were analyzed using a mixed model, with 'Ecosystem' as a random factor.

Fig. S5 Result of the leave-one-out analysis testing the robustness of our findings.

Fig. S6 Effect sizes of N addition on plant aboveground net primary productivity.

Fig. S7 Effect sizes of N addition on plant total biomass.

Fig. S8 Effect sizes of N addition on the fraction of belowground to total biomass.

Fig. S9 Effect sizes of N addition on soil cation concentrations across the entire dataset and within different soil pH ranges.

Fig. S10 Effect sizes of N addition on the concentrations of phosphorus and potassium in plant foliage, fine roots, and soils across the entire dataset and within different soil pH ranges.

Fig. S11 Relationships between aboveground biomass and foliar phosphorus and potassium concentrations under N enrichment conditions.

Fig. S12 Effect sizes of N addition on the N concentrations in plant foliage and fine roots across the entire dataset and within different soil pH ranges.

Fig. S13 Responses (%) of aboveground biomass and aboveground net primary productivity to N addition in different ecosystems and the associated soil pH of the experimental sites.

Fig. S14 Relationships between mean annual precipitation and the effects (%) of N addition on aboveground biomass and aboveground net primary productivity, along with the background soil pH.

Table S1 Egger's test results for publication bias in the study's findings.

Table S2 Comparison of the responses of plant biomass to N addition across soil pH ranges.

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