



Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems

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Abstract

Increased human-derived nitrogen (N) deposition to terrestrial ecosystems has resulted in widespread phosphorus (P) limitation of net primary productivity. However, it remains unclear if and how N-induced P limitation varies over time. Soil extracellular phosphatases catalyze the hydrolysis of P from soil organic matter, an important adaptive mechanism for ecosystems to cope with N-induced P limitation. Here we show, using a meta-analysis of 140 studies and 668 observations worldwide, that N stimulation of soil phosphatase activity diminishes over time. Whereas short-term N loading (≤ 5 years) significantly increased soil phosphatase activity by 28%, long-term N loading had no significant effect. Nitrogen loading did not affect soil available P and total P content in either short- or long-term studies. Together, these results suggest that N-induced P limitation in ecosystems is alleviated in the long-term through the initial stimulation of soil phosphatase activity, thereby securing P supply to support plant growth. Our results suggest that increases in terrestrial carbon uptake due to ongoing anthropogenic N loading may be greater than previously thought.

KEYWORDS

microbial biomass, nitrogen addition, nutrient stoichiometry balance, phosphorus limitation, soil nitrogen content, soil pH, soil phosphatase activity, soil phosphorus content

1 | INTRODUCTION

Humans have doubled nitrogen (N) inputs into terrestrial ecosystems over the past century by burning fossil fuels and using artificial fertilizers (Davidson, 2009; Galloway et al., 2008). Nitrogen can enter natural ecosystems through multiple routes, whereas phosphorus (P) derives mostly from deposition of mineral aerosols and weathering (Peñuelas et al., 2013; Thingstad et al., 2005). Phosphorus inputs are typically small compared with N inputs, leading to a strong stoichiometric imbalance between N and P in unmanaged terrestrial ecosystems (Crowley et al., 2012; Peñuelas et al., 2013; Reinhard et al., 2017).

Enhanced N loading generally increases plant growth, thereby potentially stimulating ecosystem C storage and mitigating climate change (LeBauer & Treseder, 2008; Schulte-Uebbing & de Vries, 2018). However, the imbalance between N and P inputs suggests that plant growth may gradually shift from N to P limitation over time (Elser et al., 2007; Li, Niu, & Yu, 2016; Peñuelas et al., 2013). Indeed, several reports show that P fertilization stimulates plant growth more strongly in ecosystems with elevated versus ambient N inputs (Elser et al., 2007; Li et al., 2016), suggesting an N-induced P limitation of primary productivity. On the other hand, N fertilization can stimulate plant growth in P-limited ecosystems (LeBauer & Treseder, 2008), and P limitation of plant growth frequently fails to develop, even after decades of N fertilization (Crowley et al., 2012; Finzi, 2009; Hyvönen et al., 2008; Lambers, Raven, Shaver, & Smith, 2008; Tatariw et al., 2018; Weand, Arthur, Lovett, Sikora, & Weathers, 2010). These contrasting results suggest the existence of unknown mechanisms that can alleviate N-induced P limitation of plant growth over time.

Plants and soil microorganisms preferentially invest metabolic resources to acquire nutrients that limit their growth (Bragg, 2012; Johnson, Wilson, Bowker, Wilson, & Miller, 2010; Marklein & Houlton, 2012). Soil phosphatases are enzymes produced by both plants and soil microorganisms to catalyze the hydrolysis of ester-phosphate bonds and phosphoric acid anhydrides, releasing orthophosphate that can be taken up across living cell membranes (Liu, Chen, Chen, Guo, & Li, 2020; Margalef et al., 2017; Vance, Uhde Stone, & Allan, 2003). The production of extracellular phosphatases is generally assumed to indicate P limitation of both plant and microbial growth (Jian et al., 2016; Marklein & Houlton, 2012; Vitousek, Porder, Houlton, & Chadwick, 2010). Short-term experiments across a wide range of terrestrial ecosystems show that N loading stimulates soil phosphatase activity and accelerates P cycling (Jian et al., 2016; Marklein & Houlton, 2012; Xiao, Chen, Jing, & Zhu, 2018). Yet it remains unclear whether N stimulation of soil phosphatase activity is persistent or temporally dynamic. To address this knowledge gap, we compiled a database of the effects of N loading on soil phosphatase activity consisting of 668 observations from 140 studies (Figure S1; Table S1). We synthesized these data using meta-analysis, specifically focusing on how the response of soil phosphatase activity to N loading changes over time.

2 | MATERIALS AND METHODS

2.1 | Data collection

We searched for peer-reviewed articles published before December 2019 (i.e., ~1900 to 2019) using Web of Science (<http://apps.webofknowledge.com/>), Google Scholar (<http://scholar.google.com/>), and China National Knowledge Infrastructure (<http://www.cnki.net/>). The keywords used for the article selection were: (a) “nitrogen loading” or “nitrogen fertilization” or “nitrogen enrichment” or “nitrogen elevated” or “nitrogen deposition” and (b) “phosphatase” or “acid phosphatase” or “alkaline phosphatase” and (c) “terrestrial” or “soil” or “land.”

Articles selected for this meta-analysis had to meet the following criteria: (a) vegetation, soil, and climatic parameters were similar for ambient and N loading treatments; (b) results came from field or common garden experiments; (c) N loading methods (rate, duration, form and frequency) were clearly reported; (d) the duration of the experiment was longer than 1 year; and (e) standard deviation (SD) and sample size were reported or could be calculated from the data presented in the publication. For factorial experiments, we only considered comparisons between control and treatments that differed solely in N loading (e.g., precipitation vs. precipitation plus N loading). Measurements from different years from the same study site were included separately. We excluded articles with: (a) incomplete information on the study site (e.g., when the coordinates, climatic variables or ecosystem type could not be determined); (b) ambiguous N loading methods (e.g., compost or slurry additions); (c) missing information on study duration. When multiple measurements were taken within the same year or growing season, we considered the measurement with the most accompanied ancillary variables or that from the peak of the growing season (Tatariw et al., 2018). Based on these criteria, 140 articles and 668 data points were selected (Figure S1); the original dataset is available from Figshare (<https://figshare.com/s/8ebfabf329c09de0277e>). When results from published sources were presented graphically, we used Grapher™ to digitize the data (<http://www.goldensoftware.com/products/grapher>).

2.2 | Climatic and environmental variables

For each study in our dataset, we recorded a wide range of environmental variables, including latitude (with a range from 4.12°S to 68.63°N), longitude (157.78°W to 129.18°E), elevation (4–3,559 m), background N deposition (0.17–13.80 g N m⁻² year⁻¹), mean annual temperature (MAT; -8.8 to 27.7°C), mean annual precipitation (MAP; 110–4,500 mm), and vegetation type (cropland, grassland, forest, shrubland and wetland). If the required data were not reported in the selected articles or other articles published by the same research group, we contacted the corresponding author. Otherwise, we obtained MAT and precipitation from the WorldClim Database (Fick & Hijmans, 2017), background N deposition from the Global

N deposition database (ORNL DAAC, 2017), and vegetation types from the Whittaker Biome Diagram (Whittaker, 1962).

2.3 | Nitrogen loading methods

Information on N loading rate ($\text{g N m}^{-2} \text{ year}^{-1}$), duration (year), frequency (times per year), and chemical forms (urea, NH_4NO_3 , and mixed N [combined inorganic and organic N loading]) were tabulated for each study. To make our results comparable to other meta-analyses on enhanced N deposition, continuous variables were divided into different subgroups as in earlier studies (Chen et al., 2017; Li et al., 2016; Liu & Greaver, 2010). For example, N loading rate was grouped by <5 , $5\text{--}15$ and $>15 \text{ g N m}^{-2} \text{ year}^{-1}$ and N loading frequency by <4 , $4\text{--}12$ and >12 times per year. To assess temporal variation in treatment effects, we made a distinction between short-term (<5 years) and long-term (≥ 5 years) studies (Chen et al., 2020; Kuebbing et al., 2018). The cutoff of 5 years aligned with the large survey of long-term research in ecology and evolution by Kuebbing et al. (2018).

2.4 | Meta-analysis

We quantified the effect of N loadings on each variable by calculating the natural log of the response ratio ($\ln R$), a metric commonly used in meta-analysis (Chen et al., 2015; Hedges, Gurevitch, & Curtis, 1999):

$$\overline{\ln R} = \ln \left(\frac{\overline{X}_N}{\overline{X}_C} \right) = \ln(\overline{X}_N) - \ln(\overline{X}_C), \quad (1)$$

where \overline{X}_N and \overline{X}_C are the arithmetic mean value of the variables in the N loading and ambient treatments, respectively.

The effects of N loading on soil phosphatase activity were evaluated by mixed-effects models using the `rma.mv` function from the R package “*metafor*” (Viechtbauer, 2010). “Study site” and “observation” were considered as random effects in the meta-analysis, because several study sites contributed more than one effect size (Chen, Luo, García-Palacios, et al., 2018). Effect sizes were weighted by the inverse of the pooled variance (V_i):

$$V_i = \frac{SD_N^2}{n_N X_N^2} + \frac{SD_C^2}{n_C X_C^2}, \quad (2)$$

where SD_N and SD_C are the standard deviations, n_N and n_C are the replicate numbers, and X_N and X_C are arithmetic values for the variables for N loading and ambient treatments, respectively. The results for the analyses on $\ln R$ were back-transformed and reported as percentage change with N loading to ease interpretation. The effects of N loading were considered significant if the 95% confidence interval (CI) did not overlap with zero.

2.5 | Model selection and correlation analysis

Mixed-effects meta-regression model selection was adopted to identify the most important predictors of the effects of N loading on soil phosphatase activity using the “*glmulti*” package in R (Calcagno & de Mazancourt, 2010). The model selection was based on maximum likelihood estimation. The importance of each predictor was computed as the sum of Akaike weights for models that included this predictor. A cutoff of 0.8 was set to differentiate between essential and non-essential predictor variables (Calcagno & de Mazancourt, 2010; Jiang, Carrizo, et al., 2019; Terrer, Vicca, Hungate, Phillips, & Prentice, 2016). To avoid possible artifacts associated with arbitrary category definitions, we included study duration, N loading rate and N loading frequency as continuous variables in the model selection analysis. We ran model selection with all available pairwise predictors (i.e., latitude, longitude, elevation, MAT, MAP, background N deposition, N loading methods [frequency, rate and duration, all as continuous variables], and vegetation type) because missing values were not allowed in the model selection analysis.

To explore the potential mechanisms underlying P limitation, we tabulated the following information from both ambient and N loading treatments from the original studies: aboveground biomass, soil pH, soil total N and P content, soil available P content, and soil microbial biomass. We checked whether these variables were correlated with treatment effects on phosphatase activity. Since most studies reported only a few of those variables, correlation analyses were separately conducted for each variable. When a variable correlated significantly with treatment effects on phosphatase activity, we repeated model selection with the subset of studies that included information on this variable.

3 | RESULTS

Averaged across all studies, N loading enhanced soil phosphatase activity by 13% (95% CI, 8%–18%, $p < .001$; Figure 1). This effect of

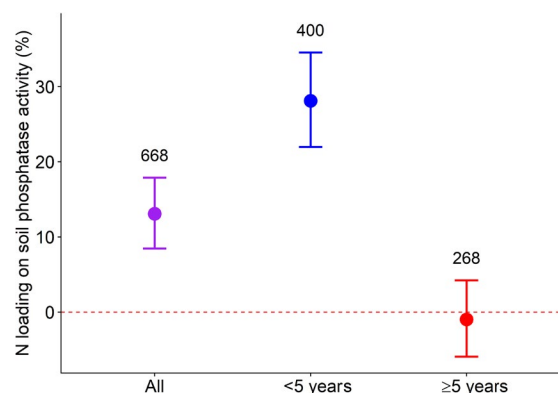


FIGURE 1 Effect of nitrogen (N) loading on soil phosphatase activity. Error bars represent bootstrap 95% confidence intervals. The sample sizes are shown above the error bars. Results are grouped by study duration for short- and long-term studies (<5 and ≥ 5 years) [Colour figure can be viewed at wileyonlinelibrary.com]

N loading was consistent regardless of vegetation type (farmland, forest, grassland, shrubland and wetland) or the rate, duration and frequency of N loading (Figures 2 and 3).

Model selection analysis of soil phosphatase activity to N loading were best explained by study duration (Figure 4). The high importance of study duration supported the removal of predictors related to climate and environmental variables (latitude, elevation, MAT, MAP and background N deposition), vegetation types and other N loading methods (Figure 4). Specifically, short-term (<5 years) N loading significantly increased soil phosphatase activity by 28% ($p < .001$), whereas long-term (≥ 5 years) N loading had no effect on soil phosphatase activity ($p = .707$; Figure 1). This differential response of soil phosphatase activity to short- and long-term N loading

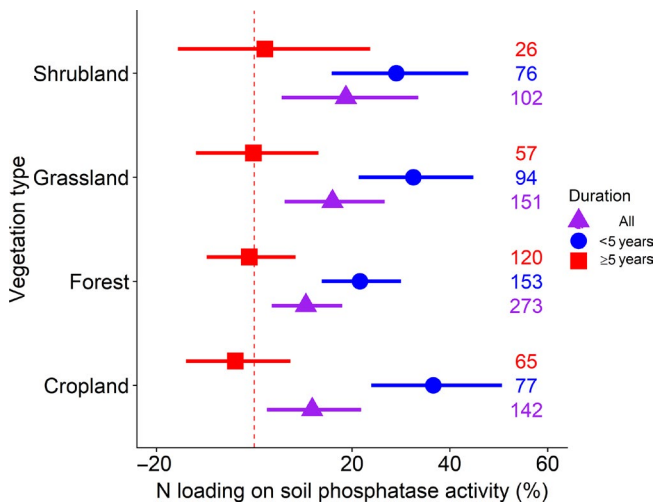


FIGURE 2 Effect of N loading on soil phosphatase activity for different vegetation types when studies are grouped by study duration. Error bars represent bootstrap 95% confidence intervals. The sample sizes are shown on the right hand of the error bars. Studies are grouped by study duration (<5 and ≥ 5 years) [Colour figure can be viewed at wileyonlinelibrary.com]

was also found within study categories based on vegetation type and N loading method (Figures 2 and 3).

Increases in soil phosphatase activity positively correlated with soil N content ($p < .001$; Figure S2a). When we repeated our model selection procedure for the subset of studies reporting soil N content, study duration remained the most important predictor of treatment effects on soil phosphatase activity (Figure S2b). Nitrogen loading did not affect soil total P or available P content in either short- or long-term studies (Figure S3a,b). The response of soil phosphatase activity was not correlated to soil total P or available P content (Figure S3c,d).

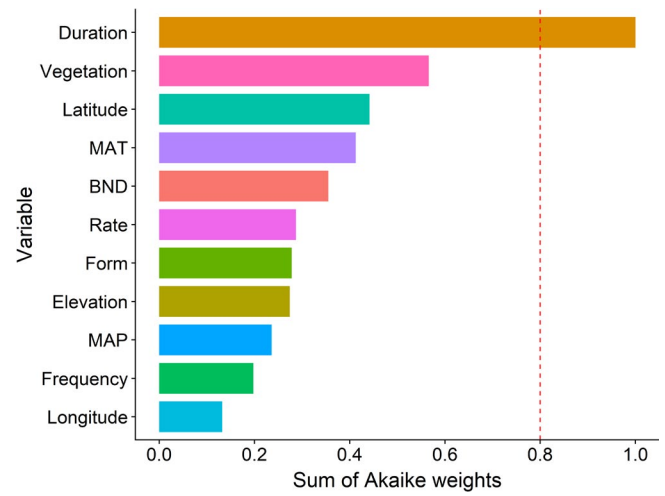


FIGURE 4 Model-averaged importance of the predictors of the effects of nitrogen (N) loading on soil phosphatase activity. Importance is estimated from the sum of Akaike weights based on model selection analysis using corrected Akaike's information criteria. Cutoff is set at 0.8 to explore the most essential variables. BND, background N deposition; MAP, mean annual precipitation; MAT, mean annual temperature. Form, duration, rate, and frequency denote different N loading methods [Colour figure can be viewed at wileyonlinelibrary.com]

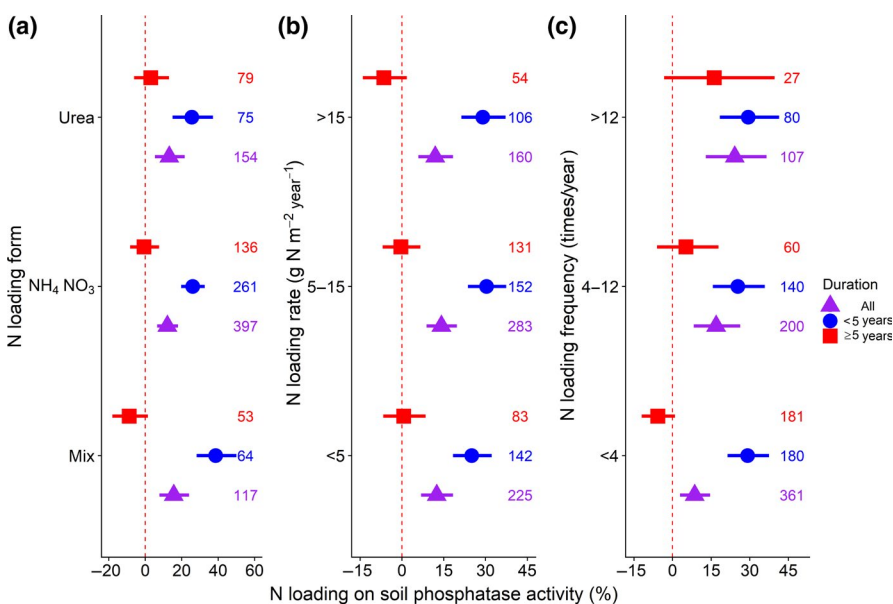


FIGURE 3 Effect of N loading on soil phosphatase activity for N loading (a) form, (b) rate and (c) frequency when studies are grouped by study duration. Error bars represent bootstrap 95% confidence intervals. The sample sizes are shown on the right hand of the error bars. Studies are grouped by study duration (<5 and ≥ 5 years) [Colour figure can be viewed at wileyonlinelibrary.com]

Nitrogen loading significantly increased aboveground biomass by 21% (95% CI, 13%–29%, $p < .001$), with no difference for short- and long-term N loading studies (Figure S4a). Nitrogen stimulation of soil phosphatase activity was positively correlated with the responses of aboveground biomass (Figure S4b). When we repeated the model selection analysis by including the response of aboveground biomass, we found that study duration and N loading rate were the most important predictors of treatment effects on soil phosphatase activity (Figure S4c).

Nitrogen loading significantly decreased soil pH by 0.24 units (95% CI, 0.17–0.32, $p < .001$; Figure S5a). The response of soil phosphatase activity to N loading did not directly relate to soil pH, but N-induced reductions in soil pH were positively correlated with N-induced changes in soil phosphatase activity (Figure S5b; $p < .001$). When we repeated the model selection analysis by including soil pH in ambient treatment and N-induced changes in soil pH, study duration remained the most important predictor of treatment effects on soil phosphatase activity (Figure S5c).

Across the dataset, N-induced changes in soil microbial biomass were positively correlated with treatment effects on soil phosphatase activity (Figure S6a). When we limited the model selection analysis to studies that reported soil microbial biomass, study duration remained the most important predictor of the effects of N loading on soil phosphatase activity (Figure S6b). In addition, short-term N loading significantly increased microbial specific phosphatase activity by 21% ($p < .001$), whereas long-term N loading had no effect (Figure S7).

4 | DISCUSSION

4.1 | Stimulation of soil phosphatase activity with N loading

Our results indicate that N loading significantly increases soil phosphatase activity across a wide range of ecosystems (Figure 2). Enhanced plant growth with N loading could drive increased demand for P, causing widespread plant and microbial P limitation (Elser et al., 2007; Li et al., 2016; Vitousek et al., 2010). This is supported by the positive relationship between N stimulation of soil phosphatase activity and aboveground biomass (Figure S4b). Plants and microbes are therefore investing energy and resources in phosphatase production, which increases the turnover rate of P-containing compounds (Bragg, 2012; Plaxton & Tran, 2011; Richardson & Simpson, 2011). Phosphatases are N-rich molecules (Midgley & Phillips, 2016; Pinsonneault, Moore, & Roulet, 2016; Sinsabaugh et al., 2008), and the additional N provides building blocks to support plant and microbial production of these enzymes (Delgado-Baquerizo et al., 2013).

4.2 | Acclimation of soil phosphatase activity to prolonged N loading

Total and available soil P content were unaffected by N loading, even in long-term studies (Figure S3a,b). Further, N stimulation of

soil phosphatase activity decreased over time (Figure 1). These results suggest that ecosystems may prevent N-induced P limitation by mechanisms that maintain P availability (discussed below), thereby weakening the initial N stimulation of soil phosphatase activity over time.

First, enhanced soil phosphatase activity during initial stages of N loading can accelerate organic P mineralization (Jian et al., 2016; Marklein & Houlton, 2012; Vitousek et al., 2010), making a portion of P bioavailable and conserved in plant biomass rather than occluded by clays and minerals (Crowley et al., 2012). The P assimilated by plants can then be re-translocated and recycled with extended N loading, e.g., by redistributing P from senescing leaves to developing tissues (Bragg, 2012).

Second, shifts in plant community composition could increase P use efficiency. Nitrogen-induced P limitation of plant growth has been observed for individual species, but is less likely for whole communities (Turner, Brenes-Arguedas, & Condit, 2018; Weand et al., 2010), suggesting that individual species differ in their response to P limitation. Species able to tolerate P limitation or those with high P-recycling capabilities will outcompete other species, alleviating the N-induced P limitation of plant growth (Farrer & Suding, 2016; Sundqvist, Liu, Giesler, & Wardle, 2014). For example, arbuscular mycorrhizal-associated herbaceous species gained a competitive advantage over ectomycorrhizal-associated larch in response to enhanced N loading in a temperate forest (Deng et al., 2016). In addition, plants can mitigate P limitations for growth through adaptation strategies related to root morphology (Castrillo et al., 2017; Gilroy & Jones, 2000; Li et al., 2020), exudation of organic acids (Lambers et al., 2008), and reduced N demands (Maathuis, 2009).

Third, N-induced microbial P limitation can increase microbial mobilization of soil P through enhanced expression of P cycling genes or through changes in microbial community composition (Carrara et al., 2018; Vance et al., 2003). For example, Chen et al. (2019) reported that long-term mineral N addition increased crop production, but decreased soil phosphatase activity. These contrasting responses were accompanied by considerable shifts in bacterial communities harboring *phoD* genes coding for PhoD alkaline phosphatases (e.g., *Stenotrophomonas* and *Brevundimonas*), showing that N addition favored bacteria with the capacity to mineralize recalcitrant organic P rather than stimulating soil phosphatase production (Chen et al., 2019; Fox, Kwapinski, Griffiths, & Schmalenberger, 2014). Similarly, N-induced changes in microbial community composition and physiology can help ecosystems adapt to P limitation (Jakobsen, Abbott, & Robson, 1992; Johnson et al., 2010; Tedersoo & Bahram, 2019; Wei et al., 2013). For example, arbuscular mycorrhizal fungi symbionts enhanced soil available P content, stimulated plant P absorption, and decreased the plant N:P ratio with N loading, which could help alleviate N-induced P limitation over time (Mei, Yang, Zhang, Zhang, & Guo, 2019; Wang et al., 2018). However, it should be noted that responses of arbuscular mycorrhizae are ecosystem specific (Cusack et al., 2016; Sekaran, McCoy, Kumar, & Subramanian, 2019; Treseder, 2008; Wang et al., 2018), and that

N loading typically decreases the abundance of arbuscular mycorrhizae (Treseder, 2004). As such, the potential of mycorrhizae to alleviate P limitation with N loading is still unclear.

Fourth, N-induced changes in soil pH could partly attenuate N-induced P limitation. Soil acidification after N loading could induce a selective pressure that drives the evolution of the soil microbial community towards preventing the N-induced P limitation of plant and microbial growth (Chen et al., 2019; Vitousek et al., 2010). In addition, N-induced soil acidification can reduce plant growth, soil C input rates, and microbial activity (Carrara et al., 2018; Chen, Luo, van Groenigen, et al., 2018), which could gradually reduce plant and microbial P requirements over time. Reductions in soil pH with long-term N loading could also help mobilize P from secondary minerals of aluminum, calcium and iron, alleviating N-induced P limitation over time (SanClements, Fernandez, & Norton, 2010; Sherman, Fernandez, Norton, Ohno, & Rustad, 2006). However, because this latter mechanism does not affect the P requirements of plants or microbes, it will not alleviate P limitation indefinitely.

Finally, prolonged N loading generally reduces belowground C allocation by plants, including decreased production of fine root biomass and root exudates (Carrara et al., 2018; Song et al., 2019). These responses can be ascribed to either N saturation over time or the accumulated deleterious effects on plant and microbial growth with long-term N loading (Aber et al., 1998; Treseder, 2008). Thus, it is likely that there is an ecosystem-specific threshold, above which prolonged N inputs do not exacerbate the P limitation, but instead reduce belowground C allocation (Tian, Wang, Sun, & Niu, 2016). Reduced belowground C allocation by plants may suppress microbial enzyme production due to C and energy limitations of microbial metabolism and growth over time (Mooshammer, Wanek, Zechmeister-Boltenstern, & Richter, 2014; Soong et al., 2019). This explanation is supported by our finding that N loading increases microbial specific phosphatase expression in the short-term, but not in the long-term. Similarly, limitation of other nutrients induced by long-term N loading (e.g., potassium, calcium and magnesium) could also constrain plant and microbial metabolism and gradually alleviate N-induced plant and microbial P limitation (Crowley et al., 2012; Wright et al., 2011).

4.3 | Soil N regulation of soil phosphatase activity with N loading

N loading increased soil phosphatase activity more strongly in ecosystems with greater soil N content (Figure S2), suggesting that plants and soil microorganisms are more prone to P limitation in N-rich ecosystems. Soil phosphatase activity can be enhanced by N loading when plant growth and microbial metabolism have shifted from N limitation to P limitation, particularly once N limitation of plant growth has been lifted (Finzi, 2009; Li et al., 2016). Since phosphatase production imposes high N costs, N loading could stimulate soil phosphatase activity more strongly in ecosystems already

with relatively high soil N content (Mineau, Fatemi, Fernandez, & Simon, 2014; Ratliff & Fisk, 2016). In ecosystems with relatively low soil N content, plant growth and microbial metabolism may remain N limited, even with enhanced N inputs (Fatemi, Fernandez, Simon, & Dail, 2016; Jones, Clode, Kilburn, Stockdale, & Murphy, 2013; Yokoyama, Imai, & Kitayama, 2017). Under these conditions, N loading would primarily support plant and microbial growth rather than the production of phosphatases (Kuzyakov & Xu, 2013; Pii et al., 2015).

4.4 | Implications

Our analysis confirms numerous studies showing that N loading leads to P limitation of both plant growth and microbial activity across a wide range of ecosystems (Elser et al., 2007; Li et al., 2016; Marklein & Houlton, 2012). However, our findings underline the importance of ecosystem processes leading to long-term acclimation of P limitation. These processes have not yet been adequately explored, and ignoring them may lead to overestimating the effect of P limitation on ecosystem function. For instance, some model simulations suggest that future P limitation of plant growth will turn ecosystems into net CO₂ sources by the end of this century (Sun et al., 2017; Wieder, Cleveland, Smith, & Todd-Brown, 2015). However, these models do not consider plant and microbial P acquisition strategies, potentially leading to substantial uncertainties in model projections (Jiang, Caldararu, Zaehle, Ellsworth, & Medlyn, 2019; Reed, Yang, & Thornton, 2015). When plant and microbial P acquisition strategies are considered, ecosystems that experience long-term enhanced N deposition can still act as net CO₂ sinks (Fleischer et al., 2019). Our results point in that direction and suggest that omitting plant and microbial P acquisition strategies may underestimate the potential of vegetation to mitigate climate change.

Phosphorus fertilization constitutes a challenge in modern agriculture, as continued P over application has led to enhanced P runoff from agricultural fields, causing eutrophication and hypoxia of lakes and marine ecosystems (Conley et al., 2009; Vance et al., 2003). Our results imply that plants and microorganisms will likely strengthen their P acquisition strategies due to N-induced P limitation, eventually leading to ecosystems being acclimated to N-induced P limitation. Indeed, soils store considerable amounts of organic P (Vance et al., 2003; Vitousek et al., 2010), which can potentially be taken up by plants and microorganisms through, for example, phosphatase catalyzed P mineralization (Margalef et al., 2017; Marklein & Houlton, 2012). Thus, by managing plant and microbial P acquisition strategies, the need for P fertilizers could potentially be reduced (Lambers et al., 2008; Menezes-Blackburn et al., 2018; Vance et al., 2003). Exploiting and applying plant and microbial P acquisition pathways with N loading is a top research priority for developing nutrient-smart and sustainable agricultural systems.

In summary, our synthesis indicates that N-induced increases in soil phosphatase activity diminish over time, even when

considering a wide range of climatic, edaphic, and experimental determinants of phosphatase activity. Our results indicate progressive attenuation of P limitation with N loading, highlighting the plant- and microbial-mediated ecosystem acclimation to N-induced P limitation. Thus, our results suggest that P limitation of plant growth with chronic N loading is smaller than previously thought. Our findings reconcile current conflicting results on N-induced P limitation, and underline the importance of ecosystem acclimation strategies to nutrient imbalances. By exploring the plant and microbial mechanisms associated with ecosystem acclimation to N-induced P limitation, we may enhance plant nutrient use efficiency and improve model predictions of net primary production in a warmer and N-enriched world.

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CONFLICT OF INTERESTS

The authors declare no competing financial interests.

AUTHOR CONTRIBUTION

J.C., K.J.v.G., and B.A.H. designed the study. J.C., C.T., S.Y., U.J., and J.E.O. collected the data. J.C., K.J.v.G., and C.T. analyzed the data. J.C., K.J.v.G., B.A.H., J.-W.v.G., F.T.M., Y.L., R.L.S., and L.E. collaborated on data interpretation. J.C., K.J.v.G., B.A.H., and C.T. wrote the manuscript. All authors contributed substantially to revisions.

DATA AVAILABILITY STATEMENT

The data associated with this paper are available from the online supplementary file or from figshare (<https://figshare.com/s/8ebfabf329c09de0277e>).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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