

DR. JI CHEN (Orcid ID : 0000-0001-7026-6312)

DR. KEES JAN VAN GROENIGEN (Orcid ID : 0000-0002-9165-3925)

DR. YIQI LUO (Orcid ID : 0000-0002-4556-0218)

Article type : Primary Research Articles

Long-term nitrogen loading alleviates phosphorus limitation in terrestrial ecosystems

Running title: Prolonged N loading eases P limitation

Ji Chen (ji.chen@agro.au.dk)^{1,2,3*}, Kees Jan van Groenigen (KJ.VanGroenigen@exeter.ac.uk)^{4*}, Bruce A. Hungate (bruce.hungate@nau.edu)⁵, César Terrer (terrermoreno1@llnl.gov)⁶, Jan-Willem van Groenigen (JanWillem.vanGroenigen@wur.nl)⁷, Fernando T. Maestre (ft.maestre@ua.es)^{8,9}, Samantha Ying (samantha.ying@ucr.edu)¹⁰, Yiqi Luo (Yiqi.Luo@nau.edu)⁵, Uffe Jørgensen (uffe.jorgensen@agro.au.dk)^{1,2}, Robert L. Sinsabaugh (rlsinsab@unm.edu)¹¹, Jørgen Eivind Olesen (jeo@agro.au.dk)^{1,3}, Lars Elsgaard (lars.elsgaard@agro.au.dk)^{1,3}

¹Department of Agroecology, Aarhus University, Tjele 8830, Denmark.

²Aarhus University Center for Circular Bioeconomy, Aarhus University, Tjele 8830, Denmark.

³iCLIMATE Interdisciplinary Centre for Climate Change, Aarhus University, Roskilde 4000, Denmark.

⁴Department of Geography, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4QG, UK.

⁵Center for Ecosystem Science and Society and Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA.

⁶Physical and Life Sciences Directorate, Lawrence Livermore National Laboratory (LLNL), Livermore, CA, USA.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/GCB.15218](https://doi.org/10.1111/GCB.15218)

This article is protected by copyright. All rights reserved

⁷Soil Biology Group, Wageningen University, Wageningen 6700AA, Netherlands.

⁸Instituto Multidisciplinar para el Estudio del Medio “Ramon Margalef”, Universidad de Alicante, Alicante 03690, Spain.

⁹Departamento de Ecología, Universidad de Alicante, Alicante 03690, Spain.

¹⁰Department of Environmental Sciences, University of California, Riverside, CA 92521, USA.

¹¹Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA.

*Corresponding author.

Ji Chen, ji.chen@agro.au.dk; Kees Jan van Groenigen, kj.vangroenigen@exeter.ac.uk (K.J.V.G.).

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 yr) 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 prevented 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.

Key words Phosphorus limitation, Nitrogen addition, Soil phosphatase activity, Soil nitrogen content, Soil phosphorus content, Soil pH, Microbial biomass, Nutrient stoichiometry balance.

1. Introduction

Humans have doubled nitrogen (N) inputs into terrestrial ecosystems over the past century by burning fossil fuels and using artificial fertilizers (Galloway *et al.*, 2008; Davidson 2009). Nitrogen can enter natural ecosystems through multiple routes, whereas phosphorus (P) derives mostly from deposition of mineral aerosols and weathering (Thingstad *et al.*, 2005; Peñuelas *et al.*, 2013). 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; Peñuelas *et al.*, 2013; Li *et al.*, 2016).

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 a 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 (Hyvönen *et al.*, 2008; Lambers *et al.*, 2008; Finzi 2009; Weand, Arthur, Lovett, Sikora, & Weathers, 2010; Crowley *et al.*, 2012; Tatariw *et al.*, 2018). 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 (Johnson, Wilson, Bowker, Wilson, & Miller, 2010; Bragg 2012; 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 (Vance, Uhde Stone, & Allan, 2003; Margalef *et al.*, 2017; Liu, Chen, Chen, Guo, Li, 2020). The production of extracellular phosphatases is generally assumed to indicate P limitation of both plant and microbial growth

(Vitousek, Porder, Houlton, & Chadwick, 2010; Marklein, & Houlton 2012; Jian *et al.*, 2016). Short-term experiments across a wide range of terrestrial ecosystems show that N loading stimulates soil phosphatase activity and accelerates P cycling (Marklein, & Houlton 2012; Jian *et al.*, 2016; 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 (Fig. S1 and 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: (i) “nitrogen loading” or “nitrogen fertilization” or “nitrogen enrichment” or “nitrogen elevated” or “nitrogen deposition” and (ii) “phosphatase” or “acid phosphatase” or “alkaline phosphatase” and (iii) “terrestrial” or “soil” or “land”.

Articles selected for this meta-analysis had to meet the following criteria: (1) vegetation, soil, and climatic parameters were similar for ambient and N loading treatments; (2) results came from field or common garden experiments; (3) N loading methods (rate, duration, form and frequency) were clearly reported; (4) the duration of the experiment was longer than one year; and (5) 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 versus precipitation plus N loading). Measurements from different years from the same study site were included separately. We excluded articles with: (i) incomplete information on the study site (e.g., when the coordinates, climatic variables or ecosystem type could not be determined); (ii) ambiguous N loading methods (e.g., compost or slurry additions); (iii) 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 (Fig. 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 to 3559 m), background N deposition (0.17 to 13.80 g N m⁻² yr⁻¹), mean annual temperature (-8.8 to 27.7°C), mean annual precipitation (110 to 4500 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 mean annual temperature 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 (g N m⁻² yr⁻¹), duration (year), frequency (times per year), and chemical forms (urea, NH₄NO₃, 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 (Liu, & Greaver 2010; Li *et al.*, 2016; Chen *et al.*, 2017). For example, N loading rate was grouped by <5, 5–15 and >15 g N m⁻² yr⁻¹ and N loading frequency by <4, 4–12 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 (Kuebbing *et al.*, 2018; Chen *et al.*, 2020). The cutoff of 5 year 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 *et al.*, 2018a). 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 cut-off of 0.8 was set to differentiate between essential and non-essential predictor variables (Calcagno, & de Mazancourt 2010; Terrer, Vicca, Hungate, Phillips, & Prentice, 2016; Jiang *et al.*, 2019). 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, mean annual temperature, mean annual precipitation, 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 to 18%, $p < 0.001$) (Fig. 1). This effect of N loading was consistent regardless of vegetation type (farmland, forest, grassland, shrubland and wetland) or the rate, duration and frequency of N loading (Figs. 2 and 3).

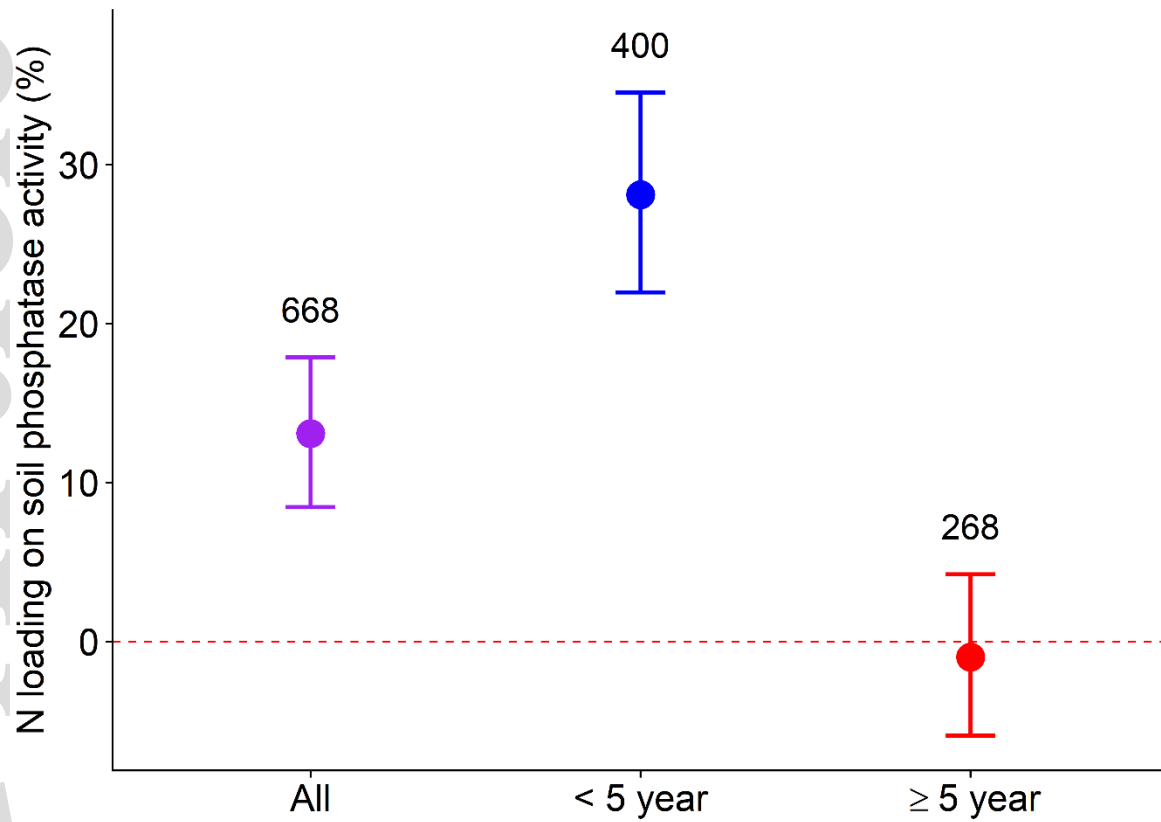


Figure 1. Effect of nitrogen (N) loading on soil phosphatase activity. Error bars represent bootstrap 95% confidence intervals. The sample sizes are shown above the error bars. Results are grouped by study duration for short- and long-term studies (<5 year and ≥ 5 year).

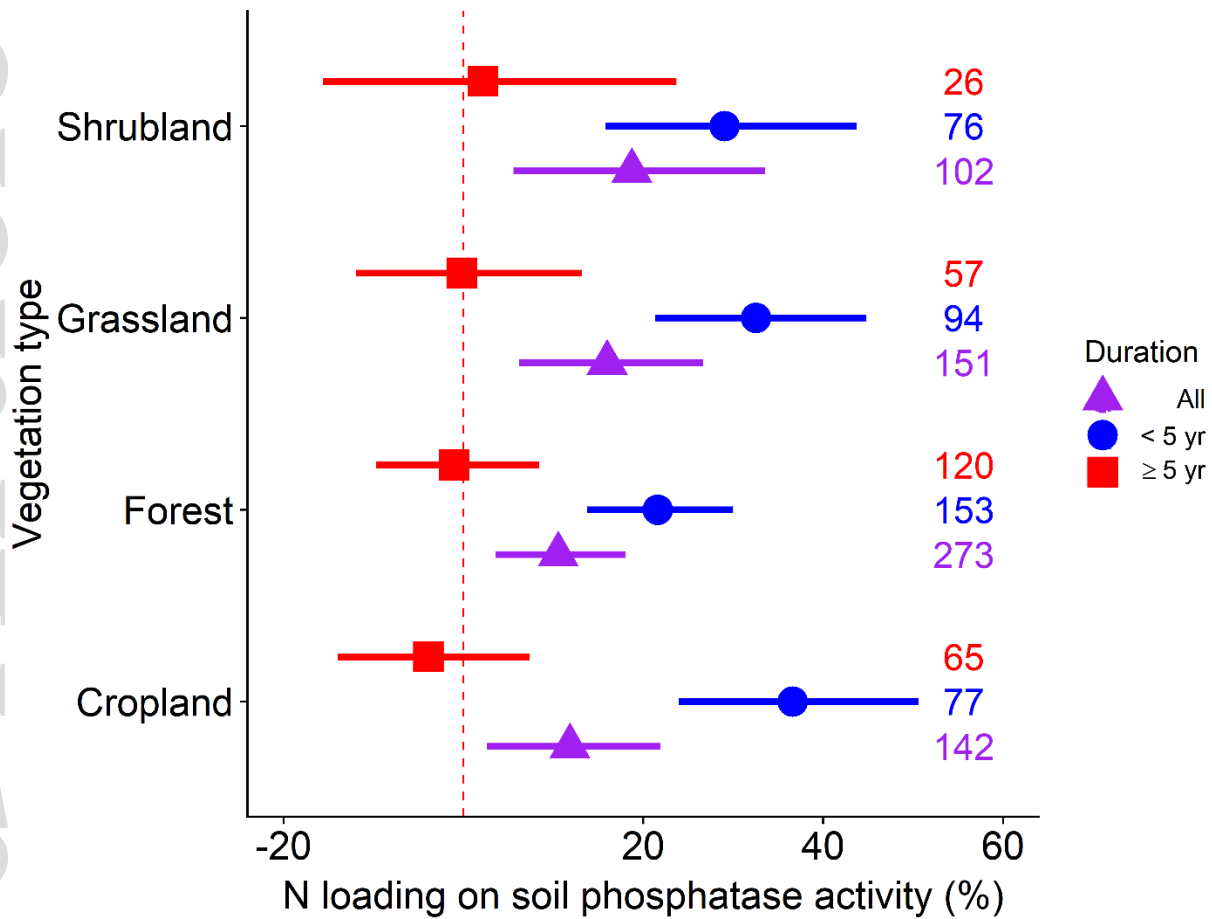


Figure 2. Effect of N loading on soil phosphatase activity for different vegetation types when studies are grouped by study duration. Error bars represent bootstrap 95% confidence intervals.

The sample sizes are shown on the right hand of the error bars. Studies are grouped by study duration (<5 year and ≥ 5 year).

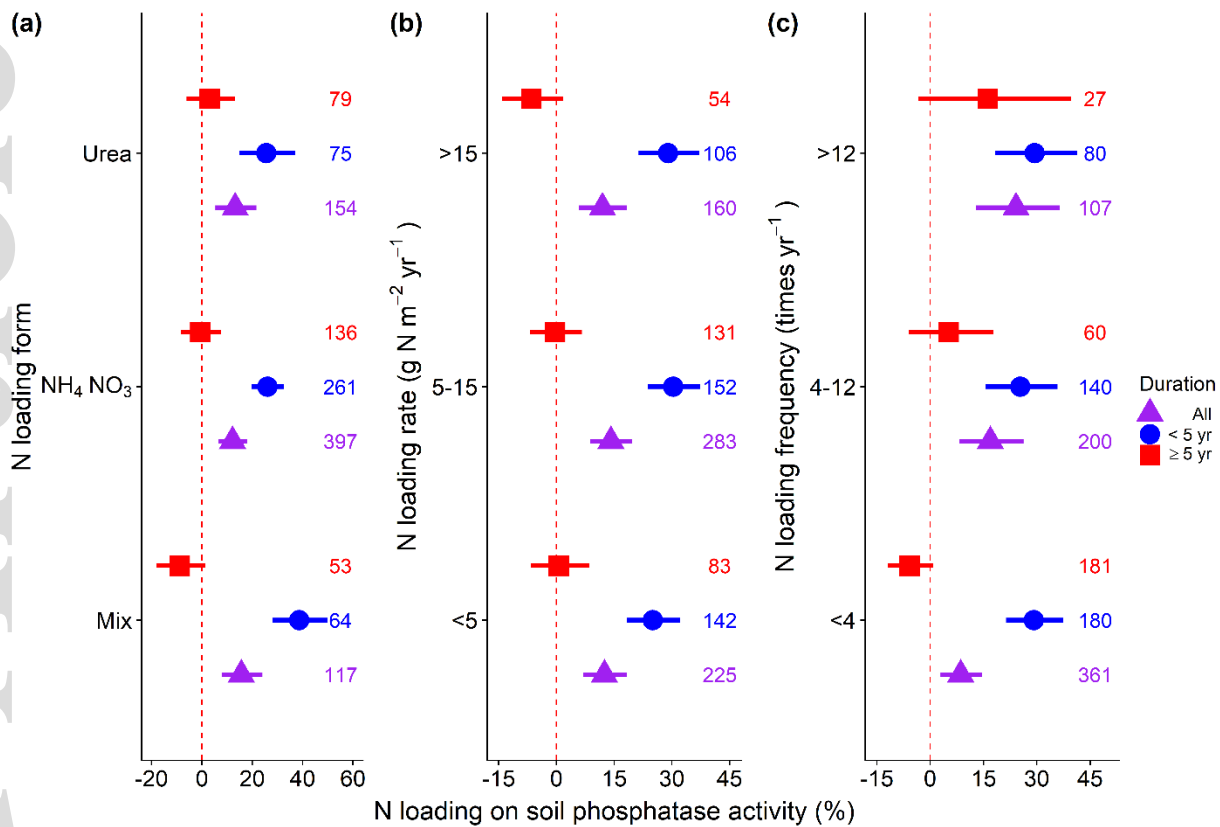


Figure 3. Effect of N loading on soil phosphatase activity for N loading (a) form, (b) rate and (c) frequency when studies are grouped by study duration. Error bars represent bootstrap 95% confidence intervals. The sample sizes are shown on the right hand of the error bars. Studies are grouped by study duration (<5 year and ≥ 5 year).

Model selection analysis of soil phosphatase activity to N loading were best explained by study duration (Fig. 4). The high importance of study duration supported the removal of predictors related to climate and environmental variables (latitude, elevation, mean annual temperature, mean annual precipitation and background N deposition), vegetation types and other N loading methods (Fig. 4). Specifically, short-term (<5 year) N loading significantly increased soil phosphatase activity by 28% ($p < 0.001$), whereas long-term (≥ 5 year) N loading had no effect on soil phosphatase activity ($p = 0.707$) (Fig. 1). This differential response of soil phosphatase activity to short- and long-term N loading was also found within study categories based on vegetation type and N loading method (Figs. 2 and 3).

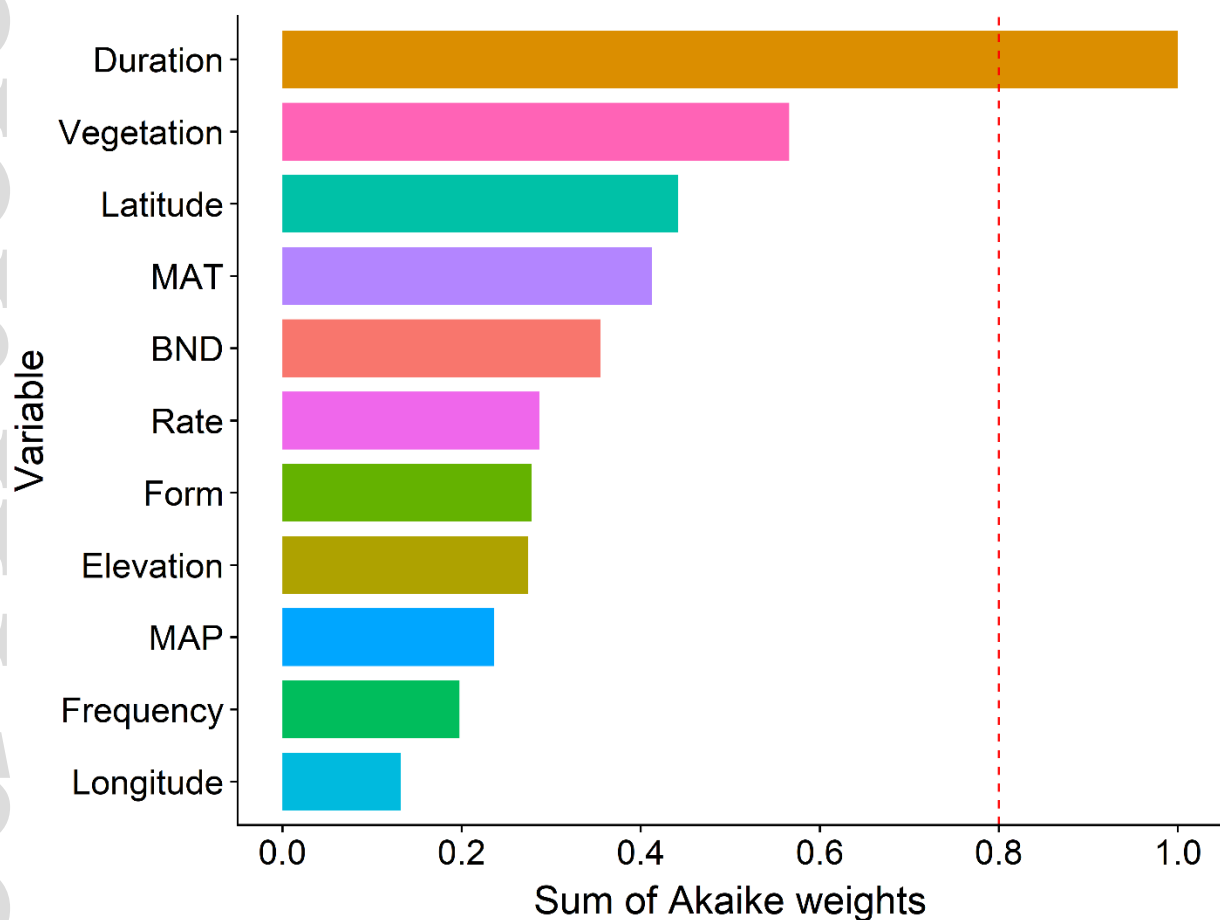


Figure 4. Model-averaged importance of the predictors of the effects of nitrogen (N) loading on soil phosphatase activity. Importance is estimated from the sum of Akaike weights based on model selection analysis using corrected Akaike's Information Criteria. Cutoff is set at 0.8 to explore the most essential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

Increases in soil phosphatase activity positively correlated with soil N content ($p < 0.001$, Fig. 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 (Fig. S2B). Nitrogen loading did not affect soil total P or available P content in either short- or long-term studies (Fig. S3A and B). The response of soil phosphatase activity was not correlated to

soil total P or available P content (Fig. S3C and D).

Nitrogen loading significantly increased aboveground biomass by 21% (95% CI, 13 to 29%, $p < 0.001$), with no difference for short- and long-term N loading studies (Fig. S4A). Nitrogen-stimulation of soil phosphatase activity was positively correlated with the responses of aboveground biomass (Fig. 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 (Fig. S4C).

Nitrogen loading significantly decreased soil pH by 0.24 units (95% CI, 0.17 to 0.32, $p < 0.001$, Fig. 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 (Fig. S5B, $p < 0.001$). 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 (Fig. S5C).

Across the dataset, N-induced changes in soil microbial biomass were positively correlated with treatment effects on soil phosphatase activity (Fig. 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 (Fig. S6B). In addition, short-term N loading significantly increased microbial specific phosphatase activity by 21% ($p < 0.001$), whereas long-term N loading had no effect (Fig. 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 (Fig. 2). Enhanced plant growth with N loading could drive increased demand for P, causing widespread plant and microbial P limitation (Elser *et al.*, 2007; Vitousek, Porder, Houlton, & Chadwick, 2010; Li *et al.*, 2016). This is supported by the positive relationship between

N-stimulation of soil phosphatase activity and aboveground biomass (Fig. S4B). Plants and microbes are therefore investing energy and resources in phosphatase production, which increases the turnover rate of P-containing compounds (Plaxton, & Tran 2011; Richardson, & Simpson 2011; Bragg 2012). Phosphatases are N-rich molecules (Sinsabaugh *et al.*, 2008; Midgley, & Phillips 2016; Pinsonneault, Moore, & Roulet, 2016), and the additional N provides the 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 (Fig. S3A and B). Further, N-stimulation of soil phosphatase activity decreased over time (Fig. 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 (Vitousek, Porder, Houlton, & Chadwick, 2010; Marklein, & Houlton 2012; Jian *et al.*, 2016), 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 (Weand, Arthur, Lovett, Sikora, & Weathers, 2010; Turner, Brenes-Arguedas, & Condit, 2018), 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 (Sundqvist, Liu, Giesler, & Wardle, 2014; Farrer, & Suding 2016). 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 (Gilroy, & Jones 2000; Castrillo *et al.*, 2017; Li *et al.*, 2020), exudation of organic acids (Lambers, Raven, Shaver, & Smith, 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 (Vance, Uhde Stone, & Allan, 2003; Carrara *et al.*, 2018). 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 (Fox, Kwapinski, Griffiths, & Schmalenberger, 2014; Chen *et al.*, 2019). Similarly, N-induced changes in microbial community composition and physiology can help ecosystems adapt to P limitation (Jakobsen, Abbott, & Robson, 1992; Johnson, Wilson, Bowker, Wilson, & Miller, 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). It should be noted though those responses of arbuscular mycorrhizae are ecosystem specific (Cusack *et al.*, 2016; Treseder 2008; Wang *et al.*, 2018; Sekaran, McCoy, Kumar, & Subramanian, 2019), 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, Porder, Houlton, & Chadwick, 2010). In addition, N-induced soil acidification can reduce plant growth, soil C input rates, and microbial activity (Carrara *et al.*, 2018;

Chen *et al.*, 2018b), 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 (Sherman, Fernandez, Norton, Ohno, & Rustad, 2006; SanClements, Fernandez, & Norton, 2010). 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 (Wright *et al.*, 2011; Crowley *et al.*, 2012).

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 (Fig. 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 (Jones, Clode, Kilburn, Stockdale, & Murphy, 2013; Fatemi, Fernandez, Simon, & Dail, 2016; 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; Marklein, & Houlton 2012; Li *et al.*, 2016). 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 (Wieder, Cleveland, Smith, & Todd-Brown, 2015; Sun *et al.*, 2017). However, these models do not consider plant and microbial P acquisition strategies, potentially leading to substantial uncertainties in model projections (Reed, Yang, & Thornton, 2015; Jiang, Caldararu, Zaehle, Ellsworth, & Medlyn, 2019). 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 (Vance, Uhde Stone, & Allan, 2003; Conley *et al.*, 2009). 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, Uhde Stone, & Allan, 2003; Vitousek,

Porder, Houlton, & Chadwick, 2010), which can potentially be taken up by plants and microorganisms through, for example, phosphatase catalyzed P mineralization (Marklein, & Houlton 2012; Margalef *et al.*, 2017). Thus, by managing plant and microbial P acquisition strategies, the need for P fertilizers could potentially be reduced (Vance, Uhde Stone, & Allan, 2003; Lambers, Raven, Shaver, & Smith, 2008; Menezes-Blackburn *et al.*, 2018). 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.

Acknowledgements We would like to appreciate the contributions from the authors whose work is included in this meta-analysis, especially those who supplied us with additional data. This study was funded by Aarhus University Centre for Circular Bioeconomy, Aarhus University Research Foundation AUFF Starting Grants (AUFF-E-2019-7-1), and Marie Skłodowska-Curie Individual Fellowship H2020-MSCA-IF-2018 (No. 839806). Ji Chen acknowledges funding support from National Natural Science Foundation of China (41701292) and China Postdoctoral Science Foundation (2017M610647, 2018T111091) when constructing the databases. César Terrer was supported by a Lawrence Fellow award through Lawrence Livermore National Laboratory (LLNL). This work was performed under the auspices of the U.S. Department of Energy by LLNL under contract DE-AC52-07NA27344 and was supported by the LLNL-LDRD Program under Project No.

20-ERD-055. Fernando T. Maestre was supported by the European Research Council (ERC Grant agreement 647038 [BIODESERT]) and Generalitat Valenciana (CIDEGENT/2018/041).

Competing financial interests The authors declare no competing financial interests.

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

Supporting Information Additional Supporting Information may be found online in the supporting information tab for this article.

ORCID

Ji Chen, <https://orcid.org/0000-0001-7026-6312>

Kees Jan van Groenigen, <https://orcid.org/0000-0002-9165-3925>

Bruce A. Hungate, <https://orcid.org/0000-0002-7337-1887>

Jan-Willem van Groenigen, <https://orcid.org/0000-0002-3162-6634>

Fernando T. Maestre, <https://orcid.org/0000-0002-7434-4856>

Samantha C. Ying, <https://orcid.org/0000-0002-1247-2529>

César Terrer, <https://orcid.org/0000-0002-5479-3486>

Jørgen Eivind Olesen, <https://orcid.org/0000-0002-6639-1273>

Lars Elsgaard, <https://orcid.org/0000-0003-0058-7609>

References

- Aber, J., McDowell, W., Nadelhoffer, K., Magill, A., Berntson, G., Kamakea, M., ... Fernandez, I. (1998). Nitrogen saturation in temperate forest ecosystems: Hypotheses revisited. *BioScience*, 48, 921-934. <https://doi.org/10.2307/1313296>
- Bragg, J. (2012). Opportunities for improving phosphorus-use efficiency in crop plants. *New Phytologist*, 195, 306-320. <https://doi.org/10.1111/j.1469-8137.2012.04190.x>
- Calcagno, V., & de Mazancourt, C. (2010). glmulti: An R package for easy automated model selection with (generalized) linear models. *Journal of Statistical Software*, 34, 1-29. <https://doi.org/10.18637/jss.v034.i12>
- Carrara, J.E., Walter, C.A., Hawkins, J.S., Peterjohn, W.T., Averill, C., & Brzostek, E.R. (2018). Interactions among plants, bacteria, and fungi reduce extracellular enzyme activities under long-term N fertilization. *Global Change Biology*, 24, 2721-2734. <https://doi.org/10.1111/gcb.14081>
- Castrillo, G., Teixeira, P.J.P.L., Paredes, S.H., Law, T.F., de Lorenzo, L., Feltcher, M.E., ... Jones, C.D. (2017). Root microbiota drive direct integration of phosphate stress and immunity. *Nature*, 543, 513-518. <https://doi.org/10.1038/nature21417>
- Chen, J., Elsgaard, L., van Groenigen, K.J., Olesen, J.E., Liang, Z., Jiang, Y., ... Jørgensen, U. (2020). Soil carbon loss with warming: New evidence from carbon-degrading enzymes. *Global Change Biology*, 26, 1944-1952. <https://doi.org/10.1111/gcb.14986>
- Chen, J., Luo, Y., García-Palacios, P., Cao, J., Dacal, M., Zhou, X., ... Groenigen, K.J. (2018a). Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. *Global Change Biology*, 24, 4816-4826. <https://doi.org/10.1111/gcb.14394>
- Chen, J., Luo, Y., Li, J., Zhou, X., Cao, J., Wang, R.-W., ... Zhou, L. (2017). Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Global Change Biology*, 23, 1328-1337. <https://doi.org/10.1111/gcb.13402>
- Chen, J., Luo, Y., van Groenigen, K.J., Hungate, B.A., Cao, J., Zhou, X., Wang, R.-W. (2018b). A keystone microbial enzyme for nitrogen control of soil carbon storage. *Science Advances*, 4, eaaq1689. <https://doi.org/10.1126/sciadv.aaq1689>
- Chen, J., Luo, Y., Xia, J., Jiang, L., Zhou, X., Lu, M., ... Cao, J. (2015). Stronger warming effects on microbial abundances in colder regions. *Scientific Reports*, 5, 18032. <https://doi.org/10.1038/srep18032>
- Chen, X., Jiang, N., Condon, L.M., Dunfield, K.E., Chen, Z., Wang, J., & Chen L. (2019). Soil alkaline phosphatase activity and bacterial phoD gene abundance and diversity under long-term nitrogen and manure inputs. *Geoderma*, 349, 36-44. <https://doi.org/10.1016/j.geoderma.2019.04.039>
- Conley, D.J., Paerl, H.W., Howarth, R.W., Boesch, D.F., Seitzinger, S.P., Havens, K.E., ... Likens, G.E. (2009). Controlling eutrophication: Nitrogen and phosphorus. *Science*, 323, 1014-1015. <https://doi.org/10.1126/science.1167755>
- Crowley, K.F., Mcneil, B.E., Lovett, G.M., Canham, C.D., Driscoll, C.T., Rustad, L.E., ... Boggs, J. L. (2012). Do nutrient

- limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? *Ecosystems*, 15, 940-957. <https://doi.org/10.1007/s10021-012-9550-2>
- Cusack, D. F., Karpman, J., Ashdown, D., Cao, Q., Ciochina, M., Halterman, S., . . . Neupane, A. (2016). Global change effects on humid tropical forests: Evidence for biogeochemical and biodiversity shifts at an ecosystem scale. *Reviews of Geophysics*, 54 (3), 523-610. <https://doi.org/10.1002/2015RG000510>
- Davidson, E.A. (2009). The contribution of manure and fertilizer nitrogen to atmospheric nitrous oxide since 1860. *Nature Geoscience*, 2, 659-662. <https://doi.org/10.1038/ngeo608>
- Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Bowker, M.A., Wallenstein, M.D., Quero, J.L., ... Zaady, E. (2013). Decoupling of soil nutrient cycles as a function of aridity in global drylands. *Nature*, 502, 672-676. <https://doi.org/10.1038/nature12670>
- Deng, M., Liu, L., Sun, Z., Piao, S., Ma, Y., Chen, Y., . . . Li, P. (2016). Increased phosphate uptake but not resorption alleviates phosphorus deficiency induced by nitrogen deposition in temperate *Larix principis-rupprechtii* plantations. *New Phytologist*, 212 (4), 1019-1029. <https://doi.org/10.1111/nph.14083>
- Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., ... Smith, J.E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10, 1135–1142. <https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Farrer, E.C., & Suding, K.N. (2016). Teasing apart plant community responses to N enrichment: The roles of resource limitation, competition and soil microbes. *Ecology Letters*, 19, 1287-1296. <https://doi.org/10.1111/ele.12665>
- Fatemi, F.R., Fernandez, I.J., Simon, K.S., & Dail, D.B. (2016). Nitrogen and phosphorus regulation of soil enzyme activities in acid forest soils. *Soil Biology and Biochemistry*, 98, 171-179. <https://doi.org/10.1016/j.soilbio.2016.02.017>
- Fick, S.E., & Hijmans, R.J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37, 4302-4315. <https://doi.org/10.1002/joc.5086>
- Finzi, A.C. (2009). Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. *Biogeochemistry*, 92, 217-229. <https://doi.org/10.1007/s10533-009-9286-z>
- Fleischer, K., Rammig, A., De Kauwe, M.G., Walker, A.P., Domingues, T.F., Fuchslueger, L., ... Lapola, D.M. (2019). Amazon forest response to CO2 fertilization dependent on plant phosphorus acquisition. *Nature Geoscience*, 12, 736-741. <https://doi.org/10.1038/s41561-019-0404-9>
- Fox, A., Kwapinski, W., Griffiths, B.S., & Schmalenberger, A. (2014). The role of sulfur- and phosphorus-mobilizing bacteria in biochar-induced growth promotion of *Lolium perenne*. *FEMS Microbiology Ecology*, 90, 78-91. <https://doi.org/10.1111/1574-6941.12374>
- Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., ... Sutton, M.A. (2008). Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science*, 320, 889-892.

<https://doi.org/10.1126/science.1136674>

- Gilroy, S., & Jones, D.L. (2000). Through form to function: Root hair development and nutrient uptake. *Trends Plant Sci.*, 5, 56-60. [https://doi.org/10.1016/S1360-1385\(99\)01551-4](https://doi.org/10.1016/S1360-1385(99)01551-4)
- Hedges, L.V., Gurevitch, J., & Curtis, P.S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150-1156. [https://doi.org/10.1890/0012-9658\(1999\)080\[1150:TMAORR\]2.0.CO;2](https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2)
- Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I., & Linder, S. (2008). Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry*, 89, 121-137. <https://doi.org/10.1007/s10533-007-9121-3>
- Jakobsen, I., Abbott, L.K., & Robson, A.D. (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. *New Phytologist*, 120, 371-380. <https://doi.org/10.1111/j.1469-8137.1992.tb01077.x>
- Jian, S., Li, J., Chen, J., Wang, G., Mayes, M.A., Dzantor, K.E., ... Luo Y. (2016). Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biology and Biochemistry*, 101, 32-43. <https://doi.org/10.1016/j.soilbio.2016.07.003>
- Jiang, M., Caldararu, S., Zaehle, S., Ellsworth, D.S., & Medlyn, B.E. (2019). Towards a more physiological representation of vegetation phosphorus processes in land surface models. *New Phytologist*, 222, 1223-1229. <https://doi.org/10.1111/nph.15688>
- Jiang, Y., Carrijo, D., Huang, S., Chen, J., Balaine, N., Zhang, W., ... Linnquist, B. (2019). Water management to mitigate the global warming potential of rice systems: A global meta-analysis. *Field Crops Research*, 234, 47-54. <https://doi.org/10.1016/j.fcr.2019.02.010>
- Johnson, N.C., Wilson, G.W.T., Bowker, M.A., Wilson, J.A., & Miller, R.M. (2010). Resource limitation is a driver of local adaptation in mycorrhizal symbioses. *Proceedings of the National Academy of Sciences*, 107, 2093-2098. <https://doi.org/10.1073/pnas.0906710107>
- Jones, D.L., Clode, P.L., Kilburn, M.R., Stockdale, E.A., & Murphy, D.V. (2013). Competition between plant and bacterial cells at the microscale regulates the dynamics of nitrogen acquisition in wheat (*Triticum aestivum*). *New Phytologist*, 200, 796-807. <https://doi.org/10.1111/nph.12405>
- Kuebbing, S.E., Reimer, A.P., Rosenthal, S.A., Feinberg, G., Leiserowitz, A., Lau, J.A., ... Bradford, M.A. (2018). Long-term research in ecology and evolution: A survey of challenges and opportunities. *Ecological Monographs*, 88, 245-258. <https://doi.org/10.1002/ecm.1289>
- Kuzyakov, Y., & Xu, X. (2013). Competition between roots and microorganisms for nitrogen: Mechanisms and ecological relevance. *New Phytologist*, 198, 656-669. <https://doi.org/10.1111/nph.12235>
- Lambers, H., Raven, J.A., Shaver, G.R., & Smith, S.E. (2008). Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution*, 23, 95-103. <https://doi.org/10.1016/j.tree.2007.10.008>

- LeBauer, D.S., & Treseder, K.K. (2008). Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology*, 89, 371-379. <https://doi.org/10.1890/06-2057.1>
- Li, Q., Chen, J., Feng, J., Wu, J., Zhang, Q., Jia, W., . . . Cheng X. (2020). How do biotic and abiotic factors regulate soil enzyme activities at plot and microplot scales under afforestation? *Ecosystems*. <https://doi.org/10.1007/s10021-019-00477-4>
- 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. <https://doi.org/10.1111/gcb.13125>
- Liu, J., Chen, J., Chen, G., Guo, J., & Li, Y. (2020). Enzyme stoichiometry indicates the variation of microbial nutrient requirements at different soil depths in subtropical forests. *Plos One*, 15 (2), e0220599. <https://doi.org/10.1371/journal.pone.0220599>
- Liu, L., & Greaver, T.L. (2010). A global perspective on belowground carbon dynamics under nitrogen enrichment. *Ecology Letters*, 13, 819-828. <https://doi.org/10.1111/j.1461-0248.2010.01482.x>
- Maathuis, F.J. (2009). Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology*, 12, 250-258. <https://doi.org/10.1016/j.pbi.2009.04.003>
- Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I., Ciais, P., ... Asensio, D. (2017). Global patterns of phosphatase activity in natural soils. *Scientific Reports*, 7, 1337. <https://doi.org/10.1038/s41598-017-01418-8>
- Marklein, A.R., & Houlton, B.Z. (2012). Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytologist*, 193, 696-704. <https://doi.org/10.1111/j.1469-8137.2011.03967.x>
- Mei, L., Yang, X., Zhang, S., Zhang, T., & Guo, J. (2019). Arbuscular mycorrhizal fungi alleviate phosphorus limitation by reducing plant N:P ratios under warming and nitrogen addition in a temperate meadow ecosystem. *Science of the Total Environment*, 686, 1129-1139. <https://doi.org/10.1016/j.scitotenv.2019.06.035>
- Menezes-Blackburn, D., Giles, C., Darch, T., George, T.S., Blackwell, M., Stutter, M., ... Haygarth, P.M. (2018). Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: A review. *Plant and Soil*, 427, 5-16. <https://doi.org/10.1007/s11104-017-3362-2>
- Midgley, M.G., & Phillips, R.P. (2016). Resource stoichiometry and the biogeochemical consequences of nitrogen deposition in a mixed deciduous forest. *Ecology*, 97, 3369-3378. <https://doi.org/10.1002/ecy.1595>
- Mineau, M.M., Fatemi, F.R., Fernandez, I.J., & Simon, K.S. (2014). Microbial enzyme activity at the watershed scale: Response to chronic nitrogen deposition and acute phosphorus enrichment. *Biogeochemistry*, 117, 131-142. <https://doi.org/10.1007/s10533-013-9869-6>
- Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., & Richter, A. (2014). Stoichiometric imbalances between terrestrial decomposer communities and their resources: Mechanisms and implications of microbial adaptations to their resources. *Frontiers in Microbiology*, 5. <https://doi.org/10.3389/fmicb.2014.00022>

ORNL DAAC. 2017. Spatial Data Access Tool (SDAT). ORNL DAAC, Oak Ridge, Tennessee, USA.

<https://doi.org/10.3334/ORN LDAAC/1388>

Peñuelas, J., Poulter, B., Sardans, J., Ciais, P., van der Velden, V.M., Bopp, L., ... Llusia, J. (2013). Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nature Communications*, 4, 1-10. <https://doi.org/10.1038/ncomms3934>

Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., & Crecchio, C. (2015). Microbial interactions in the rhizosphere: Beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biology and Fertility of Soils*, 51, 403-415. <https://doi.org/10.1007/s00374-015-0996-1>

Pinsonneault, A.J., Moore, T.R., & Roulet, N.T. (2016). Effects of long-term fertilization on peat stoichiometry and associated microbial enzyme activity in an ombrotrophic bog. *Biogeochemistry*, 129, 149-164. <https://doi.org/10.1007/s10533-016-0224-6>

Plaxton, W.C., & Tran, H.T. (2011). Metabolic adaptations of phosphate-starved plants. *Plant Physiology*, 156, 1006-1015. <https://doi.org/10.1104/pp.111.175281>

Ratliff, T.J., & Fisk, M.C. (2016). Phosphatase activity is related to N availability but not P availability across hardwood forests in the northeastern United States. *Soil Biology and Biochemistry*, 94, 61-69. <https://doi.org/10.1016/j.soilbio.2015.11.009>

Reed, S.C., Yang, X., & Thornton, P.E. (2015). Incorporating phosphorus cycling into global modeling efforts: A worthwhile, tractable endeavor. *New Phytologist*, 208, 324-329. <https://doi.org/10.1111/nph.13521>

Reinhard, C.T., Planavsky, N.J., Gill, B.C., Ozaki, K., Robbins, L.J., Lyons, T.W., ... Konhauser, K.O. (2017). Evolution of the global phosphorus cycle. *Nature*, 541, 386-389. <https://doi.org/10.1038/nature20772>

Richardson, A.E., & Simpson, R.J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology*, 156, 989-996. <https://doi.org/10.1104/pp.111.175448>

SanClements, M.D., Fernandez, I.J., & Norton, S.A. (2010). Phosphorus in soils of temperate forests: Linkages to acidity and aluminum. *Soil Science Society of America Journal*, 74, 2175-2186. <https://doi.org/10.2136/sssaj2009.0267>

Sekaran, U., McCoy, C., Kumar, S., & Subramanian, S. (2019). Soil microbial community structure and enzymatic activity responses to nitrogen management and landscape positions in switchgrass (*Panicum virgatum* L.). *Gcb Bioenergy*, 11 (7), 836-851. <https://doi.org/10.1111/gcbb.12591>

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. <https://doi.org/10.1111/gcb.13862>

Sherman, J., Fernandez, I.J., Norton, S.A., Ohno, T., & Rustad, L.E. (2006). Soil aluminum, iron, and phosphorus dynamics in response to long-term experimental nitrogen and sulfur additions at the bear brook watershed in maine, USA. *Environmental Monitoring and Assessment*, 121, 421-429. <https://doi.org/10.1007/s10661-005-9140-2>

- Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., ... Gallo, M.E. (2008). Stoichiometry of soil enzyme activity at global scale. *Ecology Letters*, 11, 1252-1264. <https://doi.org/10.1111/j.1461-0248.2008.01245.x>
- Song, J., Wan, S., Piao, S., Knapp, A.K., Classen, A.T., Vicca, S., ... Zheng, M. (2019). A meta-analysis of 1,119 manipulative experiments on terrestrial carbon-cycling responses to global change. *Nature Ecology & Evolution*, 3, 1309-1320. <https://doi.org/10.1038/s41559-019-0958-3>
- Soong, J.L., Fuchslueger, L., Marañón-Jimenez, S., Torn, M.S., Janssens, I.A., Penuelas, J., & Richter, A. (2019). Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biology*, 26, 1953-1961. <https://doi.org/10.1111/gcb.14962>
- Sun, Y., Peng, S., Goll, D.S., Ciais, P., Guenet, B., Guimberteau, M., ... Piao, S. (2017). Diagnosing phosphorus limitations in natural terrestrial ecosystems in carbon cycle models. *Earth's Future*, 5, 730-749. <https://doi.org/10.1002/2016EF000472>
- Sundqvist, M.K., Liu, Z., Giesler, R., & Wardle, D.A. (2014). Plant and microbial responses to nitrogen and phosphorus addition across an elevational gradient in subarctic tundra. *Ecology*, 95, 1819-1835. <https://doi.org/10.1890/13-0869.1>
- Tatarw, C., MacRae, J.D., Fernandez, I.J., Gruselle, M.-C., Salvino, C.J., & Simon, K.S. (2018). Chronic nitrogen enrichment at the watershed scale does not enhance microbial phosphorus limitation. *Ecosystems*, 21, 178-189. <https://doi.org/10.1007/s10021-017-0140-1>
- Tedersoo, L., & Bahram, M. (2019). Mycorrhizal types differ in ecophysiology and alter plant nutrition and soil processes. *Biological Reviews*, 94, 1854-1880. <https://doi.org/10.1111/brv.12538>
- Terrer, C., Vicca, S., Hungate, B.A., Phillips, R.P., & Prentice, I.C. (2016). Mycorrhizal association as a primary control of the CO₂ fertilization effect. *Science*, 353, 72-74. <https://doi.org/10.1126/science.aaf4610>
- Thingstad, T., Krom, M., Mantoura, R., Flaten, G.F., Groom, S., Herut, B., ... Pitta, P. (2005). Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean. *Science*, 309, 1068-1071. <https://doi.org/10.1126/science.1112632>
- Tian, D., Wang, H., Sun, J., & Niu, S. (2016). Global evidence on nitrogen saturation of terrestrial ecosystem net primary productivity. *Environmental Research Letters*, 11 (2), 024012. [10.1088/1748-9326/11/2/024012](https://doi.org/10.1088/1748-9326/11/2/024012)
- Treseder, K. K. (2004). A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO₂ in field studies. *New Phytologist*, 164 (2), 347-355. <https://doi.org/10.1111/j.1469-8137.2004.01159.x>
- Treseder, K.K. (2008). Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters*, 11, 1111-1120. <https://doi.org/10.1111/j.1461-0248.2008.01230.x>
- Turner, B.L., Brenes-Arguedas, T., & Condit, R. (2018). Pervasive phosphorus limitation of tree species but not communities in tropical forests. *Nature*, 555, 367-370. <https://doi.org/10.1038/nature25789>

- Vance, C.P., Uhde Stone, C., & Allan, D.L. (2003). Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytologist*, 157, 423-447. <https://doi.org/10.1046/j.1469-8137.2003.00695.x>
- Viechtbauer, W. (2010). Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, 36, 1-48. <https://doi.org/10.18637/jss.v036.i03>
- Vitousek, P.M., Porder, S., Houlton, B.Z., & Chadwick, O.A. (2010). Terrestrial phosphorus limitation: Mechanisms, implications, and nitrogen phosphorus interactions. *Ecological Applications*, 20, 5-15. <https://doi.org/10.1890/08-0127.1>
- Wang, C., Lu, X., Mori, T., Mao, Q., Zhou, K., Zhou, G., ... Mo, J. (2018). Responses of soil microbial community to continuous experimental nitrogen additions for 13 years in a nitrogen-rich tropical forest. *Soil Biology and Biochemistry*, 121, 103-112. <https://doi.org/10.1016/j.soilbio.2018.03.009>
- Weand, M.P., Arthur, M.A., Lovett, G.M., Sikora, F., & Weathers, K.C. (2010). The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization. *Biogeochemistry*, 97, 159-181. <https://doi.org/10.1007/s10533-009-9364-2>
- Wei, C., Yu, Q., Bai, E., Lü, X., Li, Q., Xia, J., . . . Han, X. (2013). Nitrogen deposition weakens plant–microbe interactions in grassland ecosystems. *Global Change Biology*, 19 (12), 3688-3697. <https://doi.org/10.1111/gcb.12348>
- Whittaker, R.H. (1962). Classification of natural communities. *Botanical Review*, 28, 1-239. <https://doi.org/10.1007/BF02860872>
- Wieder, W.R., Cleveland, C.C., Smith, W.K., & Todd-Brown, K. (2015). Future productivity and carbon storage limited by terrestrial nutrient availability. *Nature Geoscience*, 8, 441-444. <https://doi.org/10.1038/ngeo2413>
- Wright, S.J., Yavitt, J.B., Wurzbarger, N., Turner, B.L., Tanner, E.V.J., Sayer, E.J., ... Harms, K.E. (2011). Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology*, 92, 1616-1625. <https://doi.org/10.1890/10-1558.1>
- Xiao, W., Chen, X., Jing, X., & Zhu, B. (2018). A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry*, 123, 21-32. <https://doi.org/10.1016/j.soilbio.2018.05.001>
- Yokoyama, D., Imai, N., & Kitayama, K. (2017). Effects of nitrogen and phosphorus fertilization on the activities of four different classes of fine-root and soil phosphatases in Bornean tropical rain forests. *Plant and Soil*, 416, 463-476. <https://doi.org/10.1007/s11104-017-3225-x>