

A plant-microbe interaction framework explaining nutrient effects on primary production

Petr Čapek^{1*}, Stefano Manzoni^{2,3}, Eva Kaštovská⁴, Birgit Wild^{3,5}, Kateřina Diáková⁴, Jiří Bárta⁴, Jörg Schneckner⁶, Christina Biasi⁷, Pertti J. Martikainen⁷, Ricardo Jorge Eloy Alves⁸, Georg Guggenberger⁹, Norman Gentsch⁹, Gustaf Hugelius^{2,3}, Juri Palmtag^{2,3}, Robert Mikutta¹⁰, Olga Shibistova⁹, Tim Urich¹¹, Christa Schleper⁸, Andreas Richter^{6,12} and Hana Šantrůčková⁴

1 Pacific Northwest National Laboratory, Environmental Molecular Sciences Laboratory, Richland, WA, USA. 2 Department of Physical Geography, Stockholm University, Stockholm, Sweden. 3 Bolin Centre for Climate Research, Stockholm University, Stockholm, Sweden. 4 Department of Ecosystem Biology, University of South Bohemia, České Budějovice, Czech Republic. 5 Department of Environmental Science and Analytical Chemistry, Stockholm University, Stockholm, Sweden. 6 Department of Microbiology and Ecosystem Science, University of Vienna, Vienna, Austria. 7 Department of Environmental and Biological Sciences, University of Eastern Finland, Kuopio, Finland. 8 Department of Ecogenomics and Systems Biology, University of Vienna, Vienna, Austria. 9 Institute of Soil Science, Leibniz Universität Hannover, Hannover, Germany. 10 Soil Science and Soil Protection, Martin Luther University Halle-Wittenberg, Halle, Germany. 11 Institute of Microbiology, University of Greifswald, Greifswald, Germany. 12 Institute for Applied Systems Analysis, Ecosystems Services and Management Program, IIASA, Laxenburg, Austria. *e-mail: petr.capek@pnnl.gov

Abstract

In most terrestrial ecosystems, plant growth is limited by nitrogen and phosphorus. Adding either nutrient to soil usually affects primary production, but their effects can be positive or negative. Here we provide a general stoichiometric framework for interpreting these contrasting effects. First, we identify nitrogen and phosphorus limitations on plants and soil microorganisms using their respective nitrogen to phosphorus critical ratios. Second, we use these ratios to show how soil microorganisms mediate the response of primary production to limiting and non-limiting nutrient addition along a wide gradient of soil nutrient availability. Using a meta-analysis of 51 factorial nitrogen-phosphorus fertilization experiments conducted across multiple ecosystems, we demonstrate that the response of primary production to nitrogen and phosphorus additions is accurately predicted by our stoichiometric framework. The only pattern that could not be predicted by our original framework suggests that nitrogen has not only a structural function in growing organisms, but also a key role in promoting plant and microbial nutrient acquisition. We conclude that this stoichiometric framework offers the most parsimonious way to interpret contrasting and, until now, unresolved responses of primary production to nutrient addition in terrestrial ecosystems.

Main

The growth of terrestrial plants is globally limited by nitrogen (N) and phosphorus (P)^{1,2,3}. Adding these nutrients to the soil fosters plant growth. Across ecosystems, plant growth increases to a similar extent after addition of either N or P, suggesting widespread N and P co-limitation³. This directly contradicts Liebig's long-held and widely used Law of the Minimum, which states that the growth of an organism is constrained by the most limiting nutrient at any given time. Liebig's law was originally developed for monoculture crop systems and therefore its applicability to natural plant communities has been questioned several times^{4,5,6}. The Multiple Limiting Hypothesis offers an alternative explanation of global observations, suggesting that plants can adjust their demand and modify their environment so that N and P are equally limiting⁷. Both theoretical frameworks are widely used, but neither of them can explain the frequently observed (and counterintuitive) negative effects of nutrient addition on plant primary production^{2,3}. This negative effect has been ascribed mainly to a potential toxic effect of nutrients², but it can also occur at nutrient concentrations below the toxicity level, raising the question of why are plants responding negatively to nutrient additions? Here we hypothesize that these contrasting patterns result from interactions between plants and soil microorganisms.

As plants grow they acquire multiple nutrients from the soil. The same pool of soil nutrients, however, is also accessed by soil microorganisms, which compete with plants for the limiting nutrients. When this competition reduces nutrient availability, primary production can be inhibited^{8,9}. Soil microorganisms are particularly strong competitors for N and P, at least in the short term, because they temporarily incorporate limiting nutrients in their biomass, making them unavailable to nutrient-limited plants^{8,9,10,11}. According to the definition of nutrient limitation, when plants are limited by the availability of a certain nutrient, adding that nutrient to the soil increases plant growth. In contrast, adding a nutrient that is not limiting for plants may have opposite consequences. It may stimulate soil microorganisms to compete with plants for limiting nutrient and ultimately intensify plant nutrient limitation.

Alternatively, the activity of soil microorganisms can improve plant nutritional status when soil microorganisms mineralize organically bound nutrients that are in relative excess to other nutrients^{12,13,14,15}. In addition, direct cooperative (symbiotic) plant-microbe relationships are dominant in many ecosystems^{16,17}. Several types of symbiotic plant-microbe relationships have evolved, including arbuscular mycorrhiza, ectomycorrhiza, ericoid mycorrhiza and cooperation with dark septate endophytes or N₂-fixing bacteria. Cooperation usually incurs a trade of resources between plants and soil microorganisms¹⁸. Plants supply their microbial symbionts with labile carbon and receive nutrients in return, thus alleviating plant nutrient limitation. In a system where plant-microbe

cooperation dominates, adding a nutrient that does not limit plant growth can improve plant nutrition and growth indirectly by stimulating the cooperative soil microorganisms that are limited, contrary to plants, by the added nutrient. Thus, in comparison to systems dominated by the competitive plant-microbe relationships, plants appear to be nutrient co-limited.

Based on these arguments, we hypothesize that considering the activity of the soil microorganisms and the level of cooperation with plants may explain the observed contrasting responses of primary production to nutrient addition. Here we provide a general conceptual framework of plant-microbe interactions based on two basic assumptions: (i) plants and microorganisms differ in their stoichiometric demand for soil nutrients (N and P) and (ii) the system of interest is either dominated by competitive or by cooperative relationship between plants and soil microorganisms. Our framework predicts contrasting plant responses to an addition of N or P, depending on the dominant type of plant-microbe relationship (that is, competitive or cooperative), the N/P ratio of soil nutrients and soil organic carbon availability (Table 1). Based on published plant and microbial N/P ratios, we first identify conditions of N or P limitations for plants and microorganisms. Second, using data from 51 factorial N-P fertilization experiments conducted in a range of ecosystems, we test specific hypotheses generated by our stoichiometric framework.

Table 1 | Definition of combinations of soil conditions and plant-microbe relationships and three response groups (positive +, negative -, no ~) used in the meta-analysis

Soil conditions	Plant-microbe relationship	Fertilizer	Expected response	Observed response
N-limited plants and soil microorganisms	Cooperative	N	+	+
		P	-	-
	Competitive	N	+	+
		P	-	-
N-limited plants and P-limited soil microorganisms	Cooperative	N	+	+
		P	+	+
	Competitive	N	+	+
		P	-	-
P-limited plants and soil microorganisms	Cooperative	N	-	+*
		P	+	+
	Competitive	N	-	+*
		P	+	+
C-limited soil microorganisms	Competitive	N	+	+
		P	+	+

Observations are grouped according to respective N/P_{SOIL} , plant and microbial N/P_{CR} , soil C availability and the plant-microbe relationship, or according to expected positive, negative or no response of primary production to N and P fertilization. *Expected response of primary production to N fertilization (- or ~) does not correspond with published data (+). After revising our hypothesis (positive effect of N fertilization on P acquisition), the observed response became consistent with the expectations (+) (see Results section).

A general conceptual framework of plant-microbe interactions

Defining plant and microbial nutrient limitation

Nutrient limitation occurs when the organism's growth is constrained by the availability of nutrients in the environment. When considering only two nutrients, nutrient limitation can be conveniently defined as the relative demand for one nutrient compared to the other (Fig. 1a)¹⁹. The relative amount of available soil N and P is therefore expressed as the molar ratio between available N and P in the soil (N/P_{SOIL}), and the organism's demand for these nutrients is expressed as the N/P critical ratio (N/P_{CR})^{20,21}. In the following, $N/P_{plantCR}$ / $P_{plantCR}$ denotes the plant N/P_{CR} and N/P_{micCR} / P_{micCR} denotes the N/P_{CR} of the soil microbial community. Note that we consider N solely as a nutrient acquired by organisms for biomass production; not for the energy-related metabolism of certain soil microorganisms (for example, during nitrification, denitrification or anaerobic ammonia oxidation) or for production of extracellular enzymes.

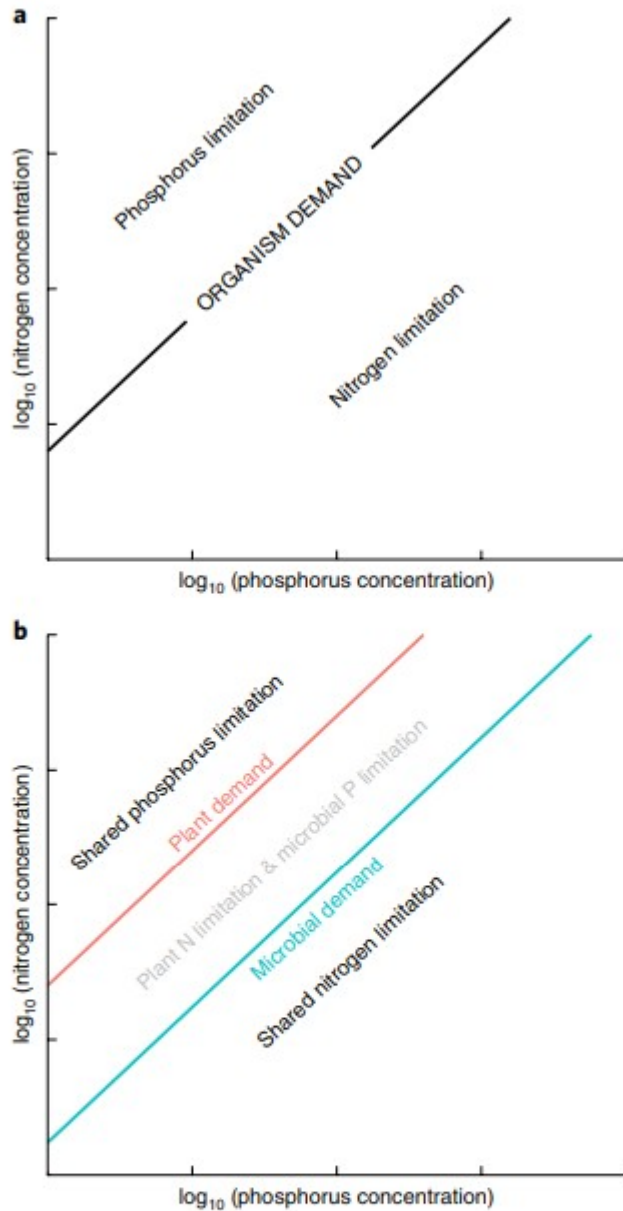


Fig. 1 | Concept of nutrient limitation. a, Concept of critical N/P molar ratio (N/P_{CR}), which reflects the organism's demands for N relative to P. An organism is N-limited if the N/P molar ratio of soil nutrients (N/P_{SOIL}) is below N/P_{CR} ; in contrast, an organism is P-limited if $N/P_{SOIL} > N/P_{CR}$. **b,** Conceptual scheme of plant-microbe interactions based on different plant and microbial N/P_{CR} . Plant demand for P (in relation to N demand) is lower than microbial demand for P (Fig. 2). Therefore, plant N limitation and microbial P limitation may occur at N/P_{SOIL} between plant and microbial N/P_{CR} .

The N/P_{CR} reflects the stoichiometric demand of N relative to P of a given organism, which is governed by the organism's biomass composition in the current physiological state (assuming comparable N and P uptake

kinetics)^{20,21,22,23,24}. According to empirical studies, $N/P_{\text{plantCRN/PCRplant}}$ can be approximated by the leaf N/P ratio. Leaf growth is more nutrient-demanding than the growth of woody tissues²² and leaves perform photosynthesis, which is crucial for plant growth. We therefore use the leaf N/P ratio as a proxy for $N/P_{\text{plantCRN/PCRplant}}$ ^{22,25}. $N/P_{\text{micCRN/PCRmic}}$ is approximated by the microbial biomass N/P ratio (assuming similar N and P use efficiency of soil microorganisms). Although leaf and microbial biomass N/P ratios may not always precisely reflect plant and microbial nutrient requirements (for example, when leaf senescence or luxury nutrient consumption occur^{21,26}, see Discussion section), they are regarded as useful proxies and are thus commonly used^{22,27,28,29,30,31,32,33}.

When the organism critical ratio and N/P_{SOIL} are equal, organisms experience optimal or balanced nutrition (Fig. 1a). When N/P_{SOIL} is lower than the critical ratio, organism growth is N-limited; on the other hand when the N/P_{SOIL} is higher than the critical ratio the organism growth is P-limited (Fig. 1a). Addition of N or P to an organism whose growth is N or P-limited promotes growth and thus biomass production. Because plants and microorganisms share the same pool of soil nutrients they also experience the same N/P_{SOIL} . Their nutrient demand, however, differs (Figs. 1b and 2a). As illustrated in Fig. 2a, $N/P_{\text{plantCRN/PCRplant}}$ is generally higher than $N/P_{\text{micCRN/PCRmic}}$ along a gradient of N/P_{SOIL} . Thus, plants require more N per unit of P than soil microorganisms and can be N-limited when soil microorganisms are P-limited.

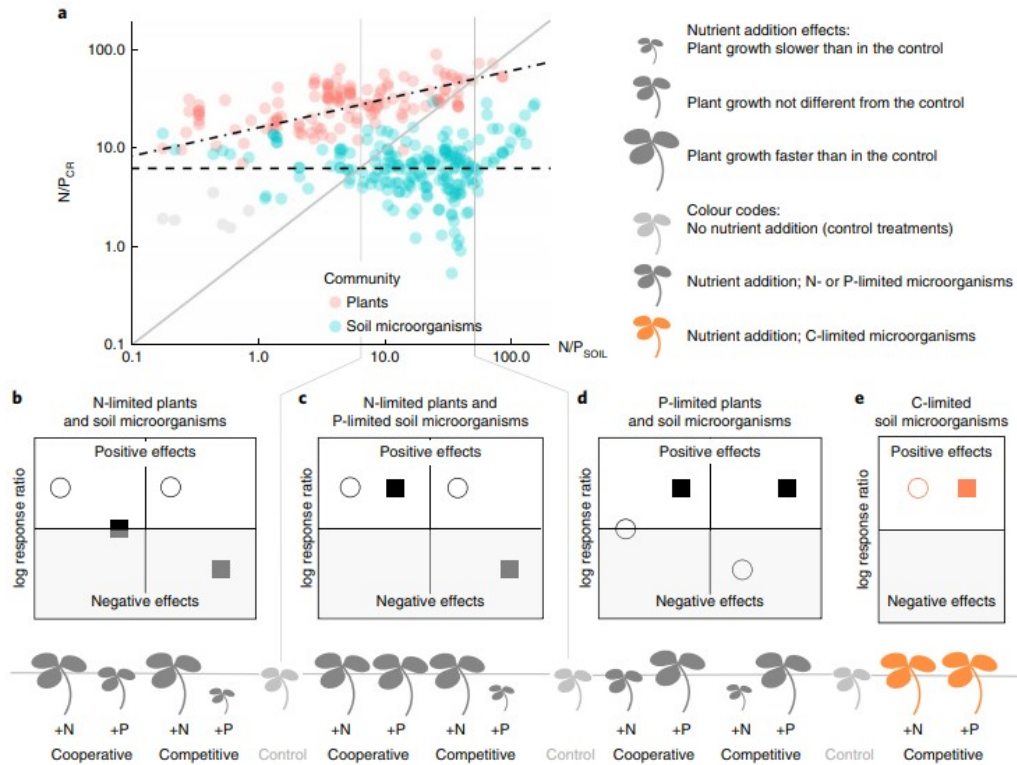


Fig. 2 | Consequences of differential plant and microbial nutrient limitations. **a**, The relationship between critical N/P ratio (N/P_{CR}) of plants (red points, $n=134$, data collected within this study) and soil microbial communities (blue points, $n=199$, data published by Manzoni et al.⁵⁶ and Xu et al.⁵⁷), and soil N/P ratio (N/P_{SOIL}). The grey line represents the one-to-one relationship; the black dashed line represents the mean microbial $N/P_{CR} = 6.3$ and the black dot-dashed line represents linear relationship between N/P_{SOIL} and plant N/P_{CR} ; $\log_{10}(N/P_{CR}^{plant}) = 1.32 + 0.2 \times \log_{10}(N/P_{SOIL})$. Grey points designate six outlier values of plant N/P_{CR} (including them would not alter our conclusions). Note that both axes have a logarithmic scale. For more details, see the Supplementary Information Sections 2.1. and 2.2. **b-e**, Expected responses of primary production (calculated as log response ratios, logRR) to N (open circles) and P (closed squares) fertilization within seven categories of different soil conditions (defined in Table 1). Orange symbols illustrate the expected response ratios when competitive soil microorganisms are C-limited. The horizontal line indicates a logRR of 0, which denotes no change; logRR lower than 0 indicates a net decrease (negative effects), whereas logRR greater than 0 indicates a net increase (positive effects).

Across ecosystems $N/P_{plantCR}/P_{plantCR}$ significantly increases with increasing N/P_{SOIL} ($\log_{10}(N/P_{plantCR}) = 1.32 + 0.2 \times \log_{10}(N/P_{SOIL})$, $F_{(1,126)} = 61.2$, $P < 0.001$), while $N/P_{micCR}/P_{micCR}$ is independent of N/P_{SOIL} ($F_{(1,197)} = 0.3$, $P = 0.61$). Soil microorganisms are therefore considered P-limited at N/P_{SOIL} above $6.3 (\pm 1.0 \text{ s.e.m.})$ and plants are considered P-limited at N/P_{SOIL} above $42.4 (\pm 8.5 \text{ s.e.m.})$. Plants are N-limited between $N/P_{SOIL} = 6.3$ and 42.4 , whereas soil microorganisms are P-limited (Fig. 1b).

Expected responses of primary production to nutrient addition

Once the definition of N and P limitation of plants and soil microorganisms is established, three categories of soil conditions (N/P_{SOIL}) referring to N-P limitation of the plant-microbe system can be defined (Fig. 1b). These categories are defined by two N/P thresholds: the N/P_{SOIL} at which plants and soil microorganisms are limited by the same nutrient (Table 1 and Fig. 2b, d) and the N/P_{SOIL} at which plants are N-limited while soil microorganisms are P-limited (Table 1 and Fig. 2c). Nevertheless, these

categories do not cover all ecologically relevant soil conditions because, in addition to N and P limitation, microbial C limitation is frequently observed across ecosystems³⁴. Thus, a fourth category referring to limitation of competitive soil microorganisms by soil organic C has to be defined to cover soil conditions occurring in most ecosystems (Table 1 and Fig. 2e). Here we adopt the common approach of regarding soil microorganisms as C-limited when soil C/N ratio is lower than the microbial C/N ratio divided by their carbon use efficiency^{35,36,37} (see Methods section).

Within these four categories of specific soil conditions, mineral N and P fertilization is expected to affect primary production either directly or indirectly by altering the activity of soil microorganisms, which can cooperate or compete with plants. The expected response of primary production to N and P fertilization is illustrated in Fig. 2b-e and listed in Table 1 and Supplementary Table 14.

When soil conditions are considered N or P limiting for plants, the addition of the respective limiting nutrient should increase primary production, irrespective of the prevailing type of plant-microbe relationship. When N and P are added in reverse to plant demands (that is, the added nutrient is not the one limiting plant growth), the response of primary production depends on the activity and level of cooperation of soil microorganisms. This response can be positive (A), negative (B) or none (C):

1. A.

A positive primary production response is expected when plants benefit from the increased activity of soil microorganisms. This may occur when P fertilization increases growth and activity of P-limited cooperative soil microorganisms, which provide plants with limiting N (Fig. 2c). This mechanism is documented for symbiotic N₂ fixation^{38,39,40,41} and mycorrhizal colonization of plant roots^{42,43}, which increases with P fertilization.

Plants may also benefit from increased nutrient mineralization of C-limited, free-living competitive soil microorganisms. Under conditions of microbial C limitation, consumed organic N and P are in excess relative to C and mineralized products are taken up by plants¹². N and P fertilization is expected to increase nutrient mineralization by intensifying microbial C limitation^{13,44} (Fig. 2e), in addition to directly providing mineral nutrients to plants. This mechanism is often observed in agricultural as well as semi-natural ecosystems^{14,15,45,46,47,48,49,50}.

2. B.

A negative primary production response is expected when competitive soil microorganisms exacerbate nutrient limitation of plants. Competition arises when plants and soil microorganisms are both limited by the same nutrient. The more limiting the nutrient is in relation to other nutrients, the more effectively microorganisms acquire that nutrient from the soil at the expense of plants. For example, higher N content in microbial biomass

relative to plant N content is observed along gradients of increasing N limitation⁹. Therefore, primary production is decreased whenever nutrient addition increases competition of plants and soil microorganisms for an increasingly limiting nutrient. When plants are N-limited and soil microorganisms are P-limited (Fig. 2c), competitive microorganisms mineralize organic N, which is in excess to P, supporting plant growth. However, when P is added and N/P_{SOIL} decreases below $N/P_{\text{plantCRN}}/P_{\text{CRplant}}$ and $N/P_{\text{micCRN}}/P_{\text{CRmic}}$ (Fig. 2b, c), N becomes limiting for both plants and soil microorganisms. Microorganisms start to retain (instead of mineralising) organic N and competition for N ensues, intensifying plant N limitation. The more P is added, the more limiting N becomes (Fig. 1a), resulting in increased plant-microbe competition and reduced plant growth (Fig. 2b). The same, vice versa, applies to N addition to P-limited soil conditions (Fig. 2d).

3. C.

No primary production response is expected when plants cannot benefit from the activity of soil microorganisms and soil microorganisms do not exacerbate plant nutrient limitation. This may only occur when the non-limiting nutrient is added to a system in which plants and soil microorganisms are limited by the same nutrient and cooperation dominates plant-microbe relationships (Fig. 2b, d).

This stoichiometric framework generates several specific hypotheses, which can be statistically tested (see Table 1, Supplementary Table 14 and 23). This framework can only predict whether the response of primary production to N or P is positive, negative or none. Predicting the magnitude of positive and negative response is beyond the capabilities of this framework.

Results and discussion

Observed response of primary production to nutrient addition

Fig. 3 shows that the responses of primary production to nutrient additions observed across natural, semi-natural and agricultural ecosystems agree with our hypotheses (Fig. 2) in most cases (detailed statistical tests are reported in Supplementary Information, sections 3.3. to 3.6). In summary, although adding the nutrient-limiting plants promotes plant growth, adding the non-limiting nutrient reduces plant growth when microorganisms are in competition with plants. However, N addition to P-limited systems yielded an unexpected positive response of primary production (Figs. 2d and 3d), which can be explained by the non-structural function of N. In our framework, N is considered solely as a nutrient acquired by organisms for growth. Nevertheless, N is also a fundamental part of extracellular enzymes and, as such, its increased availability may stimulate plant P acquisition. Marklein and Houlton⁵¹ showed that N fertilization increases the activity of extracellular phosphatases of plant and microbial origin

across different ecosystems. Importantly, plant phosphatases were shown to react positively and more strongly to N fertilization than microbial phosphatases⁵¹. Thus, we surmise that with increased N availability, P-limited plants synthesize more phosphatases, thereby acquiring more organically bound P in soil. This may alleviate plant P limitation without direct contribution of cooperative or free-living soil microorganisms. It would also explain why the type of plant-microbe relationship (cooperative versus competitive) does not affect the response of P-limited plants to N fertilization (Fig. 3c). Direct evidence of this mechanism is reported in studies by Feller et al.⁵² and Keuskamp et al.⁵³, in which initially P-limited mangrove forests exhibited both increased growth and higher phosphatase activity after N fertilization.

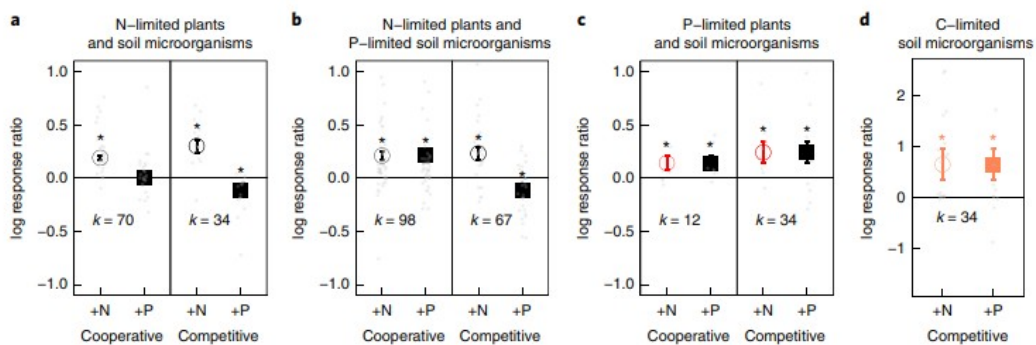


Fig. 3 | Correspondence between the theory and published data. Observed response of primary production (calculated as logRR) to N (open circles) and P (closed squares) fertilization within seven categories of different soil conditions (defined in Table 1). Orange symbols represent the response ratios when competitive soil microorganisms are C-limited. The horizontal line indicates logRR of 0, which denotes no change. logRR lower than 0 indicates a net decrease, whereas logRR greater than 0 indicates a net increase. The number of observations (k) are indicated for each category. All estimates marked by * are significantly different from zero at $P \leq 0.05$. Error bars represent standard errors. Red points indicate when observations are not in agreement with our expectations (Fig. 2). Grey dots represent the original response ratios.

These results suggest that the originally proposed hypotheses are supported in all cases, except one in which the addition of N facilitates the acquisition of P and thus indirectly promotes growth. When accounting for this indirect effect, and acknowledging that N serves for both growth and P acquisition, a full agreement between observed and expected responses of primary production is attained (Table 2).

Table 2 | Results of the linear mixed-effect model testing the significance of three different groups of observations (Table 1): expected positive, negative or no response of primary production (calculated as logRR) to N and P fertilization

	Initial hypothesis			Alternative hypothesis		
	Estimate	s.e.m.	P	Estimate	s.e.m.	P
Linear mixed-effect model coefficients						
β_0	-0.07	0.06	0.208	-0.13	0.06	0.031
β_{No}	0.10	0.08	0.202	0.15	0.09	0.078
$\beta_{Positive}$	0.35	0.06	<0.001	0.04	0.07	<0.001
AIC				426.4		421.1
I^2				96.1		96.1
Test of specific hypotheses						
Negative response	-0.07	0.06	0.208*	-0.13	0.06	0.031*
No response	0.03	0.06	0.582†	0.02	0.06	0.748†
Positive response	0.28	0.03	<0.001‡	0.28	0.03	<0.001‡

Model coefficients, corresponding standard errors, Akaike information criterion of the statistical model (AIC) and the amount of residual variability (I^2) are reported. Using the model coefficients, three hypotheses are tested (indicated by *, †, ‡). The initial hypothesis is that under P-limited conditions for plants and soil microorganisms, N fertilization decreases primary production when plants and soil microorganisms compete, and does not change primary production when plants and soil microorganisms cooperate. The alternative hypothesis is that N fertilization always increases primary production under P-limited conditions for plants and soil microorganisms (see Results section). The number of unique observations is 316 in both statistical tests. *The intercept (β_0 , **negative response**) of the mixed-effect linear model is tested against zero by the Wald-type test. †The linear combination of model coefficients β_0 and β_{No} (**no response**) is tested against zero by the Wald-type test. ‡The linear combination of model coefficients β_0 and $\beta_{Positive}$ (**positive response**) is tested against zero by the Wald-type test.

In addition to testing the hypotheses generated by our framework, we also tested additional hypotheses that could alternatively explain observed patterns in plant growth response to nutrient addition (see Supplementary Information, section 3.2). For example, negative response of plant growth to nutrient addition can be caused by the toxicity effect of the fertilizer when it is added in excessive concentrations. If that is the case, a negative correlation between the response of primary production to nutrient addition and the dose of fertilizer would be expected. Our analysis shows the exact opposite behaviour (Supplementary Table 10) suggesting that nutrient additions were under the toxicity level in most studies. It can be further argued that the response of plants to nutrient addition is ecosystem-specific²⁹ and the observed patterns are driven by differences across ecosystems. Except for strongly P-limited mangrove forests, where P addition more than triples plant growth, there was no difference in response of primary production to nutrient addition across ecosystems (Supplementary Table 12). We also tested if the duration of fertilization experiment significantly affects the measured responses, but also found no significant effect (Supplementary Table 11). Moreover, different types of fertilizers were used in the studies we considered, potentially affecting soil pH in different ways. In most studies, ammonium nitrate was used as the N fertilizer and super phosphate as the P fertilizer. The pH change after fertilization was controlled in seven studies included in our meta-analysis. In six of them, no significant change of pH after fertilization was found. In one study, soil pH significantly decreased after N and P fertilization from 5.76 to 5.28 and 5.54, respectively⁵⁴. The plant response did not follow the pH trend, but rather the predictions of the stoichiometric framework. N addition significantly increased primary production and P addition had no effect as expected in the N-limited system ($N/P_{SOIL} = 5.2$) dominated by cooperative plant-microbe relationships. Hence, these alternative

explanations do not appear to be generally valid, lending support to our proposed stoichiometric mechanisms as main drivers of plant responses to nutrient additions.

Data uncertainties

Despite the strong empirical support for our predictions, some data limitations have to be acknowledged. Our framework requires knowledge of several parameters not always measured in factorial fertilization studies. For this reason, some assumptions were made:

1. We combined studies that use different approaches to measure soil N and P. Thirty-three studies remained after including only those studies that reported extractable (rather than total) N and P, which is supposedly more directly related to N and P acquisition, but the main results did not change (Supplementary Fig. 16).

2. Plant and microbial N/P_{CR} were estimated from the N/P of leaves and soil microbial biomass, as often done^{22,27,28,29,30,31,32,33}. However, these biomass-based ratios do not always correspond well to plant and microbial nutrient requirements^{20,55}. For example, plants resorb nutrients from leaves before senescence⁵⁵, and so it could also be argued that leaves sampled shortly before senescence might not be representative of the actual nutrient demand. Microbes, on the other hand, are known to store P in the form of polyphosphates⁵⁶, which may decrease microbial N/P ratio and overestimate microbial P demand with respect to N. However, microbial P is typically measured as a flush of reactive monophosphate after soil fumigation. Therefore, polyphosphates should not be detected by the method and they are not expected to bias our estimates of microbial P demand.

3. Consistent with most biogeochemical models, microorganisms are regarded as C-limited when soil C/N is less than the microbial C/N ratio over microbial C-use efficiency (CUE)⁴⁴. When CUE and microbial biomass C/N were not reported, we assumed CUE = 0.5 and microbial C/N = 6 (Supplementary Table 1)^{36,57}. Although these values are reasonable approximations for mineral soils, both parameters might vary depending on soil C, N and P availabilities⁵⁸.

4. We classified plant-microbe systems as either competitive or cooperative based on the plant species composition. However, a range of plant-microbe relationships may emerge at any given site, and our simple classification should be interpreted as a first-order approximation capturing the tendency of a system to be predominantly competitive or cooperative.

These data limitations call for dedicated empirical approaches to further test our hypotheses, but do not weaken our general conclusions.

A stoichiometric perspective on nutrient co-limitation in plants

It is not surprising that adding N and P increases production of N- and P-limited plants, respectively. However, it is less obvious why non-limiting nutrient addition also increases primary production. Such a situation is denoted as co-limitation^{2,3}. Although several mechanisms were suggested to explain the occurrence of co-limitation in plants^{2,6,19,59,60,61} our stoichiometric framework provides an alternative explanation—the response of primary production to non-limiting nutrient addition is predicted based on the degree of nutrient limitation of soil microorganisms and on the type of plant-microbe relationship. By only using this type of information, our framework represents the most parsimonious conceptual model for understanding contrasting responses of primary production to nutrients addition. While proposing hypotheses that await testing in dedicated future fertilization experiments with a stronger focus on the stoichiometry of plant and microbial actors, our framework emphasizes some aspects of ecosystem function that are sometimes overlooked. First, the prevalence of competition versus cooperation completely alters the response to nutrient addition, with some unexpected outcomes (Fig. 3). Second, N and P cycling cannot be seen in isolation because nutrient limitations interact depending on soil nutrient status, and the availability of N can promote acquisition of P. The stoichiometric interactions have the potential to shape ecosystem functions and responses to increasingly unbalanced N/P stoichiometry in many ecosystems. Under such conditions, maintaining the cooperation between plants and soil microorganisms with inherently contrasting stoichiometric composition may be viewed as an especially advantageous strategy to preserve ecosystem functions.

Methods

Published data compilation and treatment

Plant and soil microbial community demand for N and P

Plant and microbial community demand for N and P is approximated by the N/P molar ratio of leaves and soil microbial biomass, respectively. From the studies included in the meta-analysis, we extracted measured leaf total N and P concentrations and corresponding total or extractable soil N and P concentrations. The soil N and P was usually reported for the top 15 cm of soil. In some studies, the whole soil profile of N and P concentrations were measured; however, for consistency we used only the data from the top 10–15 cm of soil.

Soil microbial biomass N and P concentrations and corresponding total soil N and P concentrations were extracted from two global data syntheses^{58,62}.

N–P factorial fertilization experiments

A preliminary list of N–P fertilization studies was extracted from the studies of LeBauer and Treseder¹ and Harpole et al.². Fertilization studies were also searched on the Web of Science and PubMed using the keywords ‘addition’, ‘fertilization’, ‘nitrogen’, ‘phosphorus’, ‘soil’, ‘co-limitation’ and their

combinations (Supplementary Fig. 1). We restricted our analysis to studies in which soil N and P were measured in unfertilized (control) soil in order to calculate initial N/P_{SOIL} (Supplementary Table 1). There are many methods to measure different pools of soil N and P. Only rarely are all N and P pools measured, and we therefore considered studies reporting both N and P for at least one pool (for example, inorganic pool or total organic pool). However, we made sure that the measured pools of both elements were conceptually equivalent in order to calculate a meaningful N/P_{SOIL} . For example, N is often measured as total soil N, but P is often measured as 'extractable P'. In this case, the N/P_{SOIL} is not meaningful due to the conceptual difference between those pools. Because the number of studies fulfilling these criteria is small, no other restrictions were applied. Applying our criteria, we found 51 relevant studies (Supplementary Table 1, Supplementary Fig.

1)^{8,9,42,43,52,53,54,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80,81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97,98,99,100,101,102,103,104,105}. These studies include experiments conducted in different agricultural, semi-natural and natural ecosystems under field or controlled conditions (Supplementary Table 1). However, our conceptual framework is general and generalizable across ecosystems, and thus the primary interest of our meta-analysis was to cover a wide range of N/P_{SOIL} , irrespective of ecosystem. Relevant data were directly extracted from published studies using the program R(3.4.2)¹⁰⁶ and the package digitize(0.0.4)¹⁰⁷. We extracted all relevant parameters that characterized plant biomass, plant growth, plant nutrition, soil N and P, and plant or microbial N/P_{CR} if reported.

To confront published data with predictions generated by the theoretical framework, several plant, soil and microbial characteristics have to be known for each experimental site within each study. If not reported directly, these characteristics were estimated as follows:

1. *C limitation of soil microbial community*. C limitation is considered only for systems with a dominant competitive plant-microbe relationship because symbiotic soil microorganisms are directly supplied by C from plants. The occurrence of microbial C limitation was estimated by comparing measured total soil C/N and the C/N_{CR} of microbial biomass. The latter was calculated as microbial biomass $C:N/CUE$ ⁴⁴ (0.5^{36}). When microbial biomass C/N was not measured directly (in only two studies), we used the global mean value of 6^{57} (Supplementary Table 1). When the total soil C/N was lower than the C/N_{CR} , soil conditions were considered C-limited. When the soil total C/N was not reported, soil conditions were considered C-limited unless the soil type implied C abundance (for example, C-rich umbric soil horizon) or when glucose was added to the soil.

2. *Plant and microbial N/P_{CR}* . When not reported directly, any missing plant and microbial N/P_{CR} were calculated as follows (Fig. 2a):

$$\log_{10}(N/P_{CR}^{plant}) = 1.32 + 0.2 \times \log_{10}(N/P_{SOIL}) \quad \log_{10}(N/P_{CR}^{mic}) = 0.799$$

For details see Supplementary Information sections 2.1. and 2.2.

3. *Plant-microbe relationship.* We assume that plants and soil microorganisms can maintain cooperative or competitive relationships. The cooperative relationship is defined as any symbiotic relationship of soil microorganisms with plants and the competitive relationship is defined as its absence. We are aware of the fact that symbiotic associations are not always beneficial for plants and that non-symbiotic interactions between plants and soil microbes are not always competitive. However, this simple distinction between symbiotic, cooperative soil microorganisms and free-living, competitive soil microorganisms is the only objective approach to define dominant cooperative and competitive plant-microbe interaction across published studies.

The dominance of symbiotic or non-symbiotic plant-microbe associations was inferred from the composition and abundance of plant species reported for the experimental sites or from the experimental soil treatment. In all studies with glucose or fungicide addition, the plant-microbe interaction was considered to be competitive because we expect that, in the presence of high concentrations of labile C, free-living and competitive saprotrophs would dominate. In all other studies, the occurrence of symbiotic associations was inferred from the tendency of the most abundant plant species to form, or not, the symbiotic associations (following the databases of Maherali et al.¹⁰⁸ and Brundrett¹⁶). When no information on the dominant species was available, we verified the presence or absence of symbiotic associations in phylogenetically close species or at the genus or family level.

Statistical analysis

The meta-analysis of published data was conducted in the program R¹⁰⁶ using the package metafor (2.0.0)¹⁰⁹. Effect sizes were calculated as logRR and the corresponding variance was calculated from the number of replicates and standard deviation of the mean. In studies where only standard errors were reported, standard deviations were back-calculated from standard errors and number of replicates. We used all parameters extracted from studies that were related to plant growth (see Supplementary Information, section 3.1). In several studies, more than one parameter that was related to primary production was usually measured at the end of the experiment. The complete list of all measured plant parameters is reported in Supplementary Table 1. Because these parameters are positively correlated, the calculated effect sizes were aggregated using Borenstein, Hedges, Higgins and Rothstein procedure using package MAd¹¹⁰ and assuming a correlation between effect sizes, $r = 0.3$.

We used linear mixed-effect models to test the correspondence between theoretical predictions and data in two ways:

1. *Analysis of subgroups*. The dataset was divided into seven subgroups defined in Table 1 (see also Supplementary Table 14). The subgroups were defined according to measured or estimated plant and microbial N/P_{CR} , N/P_{SOIL} and potential organic C limitation. All data are found in Supplementary Table 1 and shown on Supplementary Fig. 8. For each subgroup, we fitted the linear mixed-effect model:

$$\log RR = \beta_0 + \beta_{\text{Phosphorus}}$$

in which $\log RR$ is a response of primary production (calculated as $\log RR$), β_0 is a model intercept, which determines the response of primary production to N fertilization, and $\beta_{\text{phosphorus}}$ determines the additive effect of P fertilization. We tested the statistical significance of both model coefficients and we also tested if β_0 and linear combination of $\beta_0 + \beta_{\text{phosphorus}}$ were significantly different from zero (see Supplementary Information section 3.2. for details and examples).

2. *Analysis of response groups*. Three groups of observations, determining three expected responses of primary production, were defined: 'Positive', 'Negative' and 'No' (Table 1 and Supplementary Table 23). A linear mixed-effect model of following formula was fit to the whole dataset:

$$\log RR = \beta_0 + \beta_{\text{No}} + \beta_{\text{Positive}}$$

In this formula, β_0 determines the response of primary production of the 'Negative' group, β_{No} determines the magnitude of additive effect for the 'No' group and β_{Positive} determines the magnitude of additive effect for the 'Positive' group (see Supplementary Information, section 3.6). Using this statistical model, we tested if all model coefficients were statistically significant, and also if β_0 is significantly lower than zero, $\beta_0 + \beta_{\text{No}}$ is not different from zero and $\beta_0 + \beta_{\text{Positive}}$ is significantly higher than zero.

In addition to the testing of the theoretical predictions, reasonable and less complex (more parsimonious) hypotheses were tested using the same dataset. The statistical analysis is described step-by-step in the Supplementary Information, sections 3.3. to 3.6. All statistical tests are provided as R scripts in the source code for Supplementary Information.

References

1. LeBauer, D. S. & Treseder, K. K. Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed. *Ecology* 89, 371–379 (2008).
2. Harpole, W. S. et al. Nutrient co-limitation of primary producer communities. *Ecol. Lett.* 14, 852–862 (2011).
3. Elser, J. J. et al. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142 (2007).

4. Gorban, A. N., Pokidysheva, L. I., Smirnova, E. V. & Tyukina, T. A. Law of the minimum paradoxes. *Bull. Math. Biol.* 73, 2013–2044 (2010).
5. Danger, M., Daufresne, T., Lucas, F., Pissard, S. & Lacroix, G. Does Liebig's law of the minimum scale up from species to communities? *Oikos* 117, 1741–1751 (2008).
6. Marleau, J. N., Guichard, F. & Loreau, M. Emergence of nutrient co-limitation through movement in stoichiometric meta-ecosystems. *Ecol. Lett.* 18, 1163–1173 (2015).
7. Farrior, C. E. et al. Resource limitation in a competitive context determines complex plant responses to experimental resource additions. *Ecology* 94, 2505–2517 (2013).
8. Schmidt, I. K., Michelsen, A. & Jonasson, S. Effects on plant production after addition of labile carbon to arctic/alpine soils. *Oecologia* 112, 305–313 (1997).
9. Schmidt, I. K., Michelsen, A. & Jonasson, S. Effects of labile soil carbon on nutrient partitioning between an arctic graminoid and microbes. *Oecologia* 112, 557–565 (1997).
10. Kuzyakov, Y. & Xu, X. L. Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. *New Phytol.* 198, 656–669 (2013).
11. Wild, B. et al. Amino acid production exceeds plant nitrogen demand in Siberian tundra. *Environ. Res. Lett.* 13, 034002 (2018).
12. Manzoni, S., Trofymow, J. A., Jackson, R. B. & Porporato, A. Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. *Ecol. Monogr.* 80, 89–106 (2010).
13. Spohn, M. & Kuzyakov, Y. Phosphorus mineralization can be driven by microbial need for carbon. *Soil. Biol. Biochem.* 61, 69–75 (2013).
14. Sakala, W. D., Cadisch, G. & Giller, K. E. Interactions between residues of maize and pigeonpea and mineral N fertilizers during decomposition and N-mineralization. *Soil. Biol. Biochem.* 32, 679–688 (2000).
15. Chen, Y. et al. Nitrogen mineralization as a result of phosphorus supplementation in long-term phosphate deficient soil. *Appl. Soil Ecol.* 106, 24–32 (2016).
16. Brundrett, M. C. Mycorrhizal associations and other means of nutrition of vascular plants: understanding the global diversity of host plants by resolving conflicting information and developing reliable means of diagnosis. *Plant Soil* 320, 37–77 (2009).
17. Smith, S. E. & Read, D. J. *Mycorrhizal Symbiosis* 3rd ed (Academic Press, Cambridge, MA, 2010).

18. Franklin, O. et al. Forests trapped in nitrogen limitation—an ecological market perspective on ectomycorrhizal symbiosis. *New Phytol.* 203, 657–666 (2014).
19. Ågren, G. I., Wetterstedt, J. Å. & Billberger, M. F. K. Nutrient limitation on terrestrial plant-modeling the interaction between nitrogen and phosphorus. *New Phytol.* 194, 953–960 (2012).
20. Čapek, P., Kotas, P., Manzoni, S. & Šantrůčková, H. Drivers of phosphorus limitation across soil microbial communities. *Funct. Ecol.* 30, 1705–1713 (2016).
21. Gusewell, S. N. P. ratios in terrestrial plants: variation and functional significance. *New Phytol.* 164, 243–266 (2004).
22. Elser, J. J., Fagan, W. F., Kerkhof, A. J., Swenson, N. G. & Enquist, B. J. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytol.* 186, 593–608 (2010).
23. Ågren, G. I. The C:N/P stoichiometry of autotrophs—theory and observations. *Ecol. Lett.* 7, 185–191 (2004).
24. Klausmeier, C. A., Litchman, E., Daufresne, T. & Levin, S. A. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* 429, 171–174 (2004).
25. Minden, V. & Kleyer, M. Internal and external regulation of plant organ stoichiometry. *Plant Biol.* 16, 897–907 (2014).
26. Cotner, J. B., Makino, W. & Biddanda, B. A. Temperature affects stoichiometry and biochemical composition of *Escherichia coli*. *Microb. Ecol.* 52, 26–33 (2006).
27. Cherif, M. & Loreau, M. When microbes and consumers determine the limiting nutrient of autotrophs: a theoretical analysis. *Proc. Biol. Sci.* 276, 487–497 (2009).
28. Yan, Z. B. et al. Effects of nitrogen and phosphorus supply on growth rate, leaf stoichiometry, and nutrient resorption of *Arabidopsis thaliana*. *Plant Soil* 388, 147–155 (2015).
29. Makino, W., Cotner, J. B., Sterner, R. W. & Elser, J. J. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. *Funct. Ecol.* 17, 121–130 (2003).
30. Yan, J. et al. The mechanism for exclusion of *Pinus massoniana* during the succession in subtropical forest ecosystems: light competition or stoichiometric homeostasis? *Sci. Rep.* 5, 10994 (2015).
31. Hall, E. K. et al. Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. *Ecosystems* 14, 261–273 (2010).

32. Zechmeister-Boltenstern, S. et al. The application of ecological stoichiometry to plant-microbial-soil organic matter transformations. *Ecol. Monogr.* 85, 133-155 (2015).
33. Gusewell, S., Gessner, M. O., Gusewell, S. & Gessner, M. O. N. P. ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. *Funct. Ecol.* 23, 211-219 (2009).
34. Demoling, F., Figueroa, D. & Baath, E. Comparison of factors limiting bacterial growth in different soils. *Soil. Biol. Biochem.* 39, 2485-2495 (2007).
35. Manzoni, S. et al. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytol.* 196, 79-91 (2012).
36. Sinsabaugh, R. L., Manzoni, S., Moorhead, D. L., Richter, A. & Elser, J. Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecol. Lett.* 16, 930-939 (2013).
37. Doi, H. et al. Integrating elements and energy through the metabolic dependencies of gross growth efficiency and the threshold elemental ratio. *Oikos* 119, 752-765 (2010).
38. Isaac, M. E., Hinsinger, P. & Harmand, J. M. Nitrogen and phosphorus economy of a legume tree-cereal intercropping system under controlled conditions. *Sci. Total Environ.* 434, 71-78 (2012).
39. Augusto, L., Delerue, F., Gallet-Budynek, A. & Achat, D. L. Global assessment of limitation to symbiotic nitrogen fixation by phosphorus availability in terrestrial ecosystems using a meta-analysis approach. *Global Biogeochem. Cycles* 27, 804-815 (2013).
40. Diáková, K. et al. Variation in N₂ fixation in subarctic tundra in relation to landscape position and nitrogen pools and fluxes. *Arctic Antarct. Alp. Res.* 48, 111-125 (2016).
41. Šantrůčková, H., Rejmánková, E., Pivničková, B. & Snyder, J. M. Nutrient enrichment in tropical wetlands: shifts from autotrophic to heterotrophic nitrogen fixation. *Biogeochemistry* 101, 295-310 (2010).
42. Lagrange, A., L'Huillier, L. & Amir, H. Mycorrhizal status of Cyperaceae from New Caledonian ultramafic soils: effects of phosphorus availability on arbuscular mycorrhizal colonization of *Costularia comosa* under field conditions. *Mycorrhiza* 23, 655-661 (2013).
43. Koide, R. T., Huenneke, L. F., Hamburg, S. P. & Mooney, H. A. Effects of applications of fungicide, phosphorus and nitrogen on the structure and productivity of an annual serpentine plant community. *Funct. Ecol.* 2, 335 (1988).
44. Mooshammer, M. et al. Adjustment of microbial nitrogen use efficiency to carbon: nitrogen imbalances regulates soil nitrogen cycling. *Nat. Commun.* 5, 3694 (2014).

45. Sagggar, S., Parfitt, R. L., Salt, G. & Skinner, M. F. Carbon and phosphorus transformations during decomposition of pine forest floor with different phosphorus status. *Biol. Fertil. Soils* 27, 197–204 (1998).
46. Dietrich, K., Spohn, M., Villamagua, M. & Oelmann, Y. Nutrient addition affects net and gross mineralization of phosphorus in the organic layer of a tropical montane forest. *Biogeochemistry* 136, 223–236 (2017).
47. Nave, L. E., Vance, E. D., Swanston, C. W. & Curtis, P. S. Impacts of elevated N inputs on north temperate forest soil C storage, C/N, and net N-mineralization. *Geoderma* 153, 231–240 (2009).
48. Hatch, D. J., Lovell, R. D., Antil, R. S., Jarvis, S. C. & Owen, P. M. Nitrogen mineralization and microbial activity in permanent pastures amended with nitrogen fertilizer or dung. *Biol. Fertil. Soils* 30, 288–293 (2000).
49. Johnson, D. W., Edwards, N. T. & Todd, D. E. Nitrogen mineralization, immobilization, and nitrification following urea fertilization of a forest soil under field and laboratory conditions. *Soil Sci. Soc. Am. J.* 44, 610 (1980).
50. Adams, M. A. & Attiwill, P. M. Patterns of nitrogen mineralization in 23-year old pine forest following nitrogen fertilizing. *For. Ecol. Manage.* 7, 241–248 (1984).
51. Marklein, A. R. & Houlton, B. Z. Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol.* 193, 696–704 (2012).
52. Feller, I. C., McKee, K. L., Whigham, D. F. & O'Neill, J. P. Nitrogen vs. phosphorus limitation across an ecotonal gradient in a mangrove forest. *Biogeochemistry* 62, 145–175 (2003).
53. Keuskamp, J. A., Feller, I. C., Laanbroek, H. J., Verhoeven, J. T. A. & Hefing, M. M. Short- and long-term effects of nutrient enrichment on microbial exoenzyme activity in mangrove peat. *Soil. Biol. Biochem.* 81, 38–47 (2015).
54. Watanabe, T., Urayama, M., Shinano, T., Okada, R. & Osaki, M. Application of ionomics to plant and soil in fields under long-term fertilizer trials. *+* 4, 781 (2015).
55. Chang, Y. et al. Nutrients resorption and stoichiometry characteristics of different-aged plantations of *Larix kaempferi* in the Qinling Mountains, central China. *PLoS ONE* 12, e0189424 (2017).
56. Kulaev, I., Vagabov, V. & Kulakovskaya, T. New aspects of inorganic polyphosphate metabolism and function. *J. Biosci. Bioeng.* 88, 111–129 (1999).
57. Xu, X. et al. Convergence of microbial assimilations of soil carbon, nitrogen, phosphorus, and sulfur in terrestrial ecosystems. *Sci. Rep.* 5, 17445 (2015).

58. Manzoni, S. et al. Optimal metabolic regulation along resource stoichiometry gradients. *Ecol. Lett.* 20, 1182–1191 (2017).
59. Olde Venterink, H. Productivity increase upon supply of multiple nutrients in fertilization experiments; co-limitation or chemical facilitation? *Plant Soil* 408, 515–518 (2016).
60. Bracken, M. E. S. et al. Signatures of nutrient limitation and co-limitation: responses of autotroph internal nutrient concentrations to nitrogen and phosphorus additions. *Oikos* 124, 113–121 (2015).
61. Dutta, P. S., Kooi, B. W. & Feudel, U. Multiple resource limitation: nonequilibrium coexistence of species in a competition model using a synthesizing unit. *Teor. Ecol.* 7, 407–421 (2014).
62. Xu, X. F., Tornton, P. E. & Post, W. M. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* 22, 737–749 (2013).
63. Wassen, M., Van Der Vliet, R. & Verhoeven, J. Nutrient limitation in the Biebrza fens and foodplain (Poland). *ACTA Bot. Neerl.* 47, 241–253 (1998).
64. Wright, S. J. et al. Potassium, phosphorus, or nitrogen limit root allocation, tree growth, or litter production in a lowland tropical forest. *Ecology* 92, 161–1625 (2011).
65. Campo, J. & Vázquez-Yanes, C. Effects of nutrient limitation on aboveground carbon dynamics during tropical dry forest regeneration in Yucatán, Mexico. *Ecosystems* 7, 311–319 (2004).
66. Santiago, L. S. et al. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. *J. Ecol.* 100, 309–316 (2012).
67. Carpenter, A. T., Moore, J. C., Redente, E. F. & Stark, J. C. Plant community dynamics in a semi-arid ecosystem in relation to nutrient addition following a major disturbance. *Plant Soil* 126, 91–99 (1990).
68. Vitousek, P. M., Walker, L. R., Whiteaker, L. D. & Matson, P. A. Nutrient limitations to plant growth during primary succession in Hawaii Volcanoes National Park. *Biogeochemistry* 23, 197–215 (1993).
69. Batterman, S. A., Wurzburger, N. & Hedin, L. O. Nitrogen and phosphorus interact to control tropical symbiotic N₂ fixation: a test in *Inga punctata*. *J. Ecol.* 101, 1400–1408 (2013).
70. Limpens, J., Berendse, F. & Klees, H. How phosphorus availability affects the impact of nitrogen deposition on sphagnum and vascular plants in bogs. *Ecosystems* 7, 793–804 (2004).
71. Zamin, T. J., Bret-Harte, M. S. & Grogan, P. Evergreen shrubs dominate responses to experimental summer warming and fertilization in Canadian mesic low arctic tundra. *J. Ecol.* 102, 749–766 (2014).

72. Lammerts, E. J., Pegtel, D. M., Grootjans, A. P. & van der Veen, A. Nutrient limitation and vegetation changes in a coastal dune slack. *J. Veg. Sci.* 10, 111–122 (1999).
73. Zhu, F., Lu, X., Mo, J. & Ej, P. Phosphorus limitation on photosynthesis of two dominant understory species in a lowland tropical forest. *J. Plant Ecol.* 7, 526–534 (2014).
74. Vitousek, P. M. & Farrington, H. Nutrient limitation and soil development: experimental test of a biogeochemical theory. *Biogeochemistry* 37, 63–75 (1997).
75. Augustine, D. J., McNaughton, S. J. & Frank, D. A. Feedbacks between soil nutrients and large herbivores in a managed savanna ecosystem. *Ecol. Appl.* 13, 1325–1337 (2003).
76. Alvarez, R., Santanoglia, O. J. & Garcia, R. Effect of temperature on soil microbial biomass and its metabolic quotient in situ under different tillage systems. *Biol. Fertil. Soils* 19, 227–230 (1995).
77. Chen, F. -S., Zeng, D. -H., Fahey, T. J., Yao, C. -Y. & Yu, Z. -Y. Response of leaf anatomy of *Chenopodium acuminatum* to soil resource availability in a semi-arid grassland. *Plant Ecol.* 209, 375–382 (2010).
78. Mayor, J. R., Mack, M. C. & Schuur, E. A. G. Decoupled stoichiometric, isotopic, and fungal responses of an ectomycorrhizal black spruce forest to nitrogen and phosphorus additions. *Soil. Biol. Biochem.* 88, 247–256 (2015).
79. Van Duren, I. C., Boeye, D. & Grootjans, A. P. Nutrient limitations in an extant and drained poor fen: implications for restoration. *Plant Ecol.* 133, 91–100 (1997).
80. Haag, R. W. Nutrient limitations to plant production in two tundra communities. *Can. J. Bot.* 52, 103–116 (1974).
81. Sundqvist, M. K., Liu, Z., Giesler, R. & Wardle, D. A. Plant and microbial responses to nitrogen and phosphorus addition across an elevational gradient in subarctic tundra. *Ecology* 95, 1819–1835 (2014).
82. van der Hoek, D., van Mierlo Anita, J. E. M. & van Groenendael, J. M. Nutrient limitation and nutrient-driven shifts in plant species composition in a species-rich fen meadow. *J. Veg. Sci.* 15, 389–396 (2004).
83. Bowman, W. D., Teodose, T. A., Schardt, J. C. & Conant, R. T. Constraints of nutrient availability on primary production in two alpine tundra communities. *Ecology* 74, 2085–2097 (1993).
84. Davidson, E. A. et al. Nitrogen and phosphorus limitation of biomass growth in a tropical secondary forest. *Ecol. Appl.* 14, 150–163 (2004).
85. Güsewell, S., Koerselman, W. & Verhoeven, J. T. A. Time-dependent effects of fertilization on plant biomass in floating fens. *J. Veg. Sci.* 13, 705–718 (2002).

86. Ngai, J. T. & Jefferies, R. L. Nutrient limitation of plant growth and forage quality in Arctic coastal marshes. *J. Ecol.* 92, 1001–1010 (2004).
87. Potthast, K., Hamer, U. & Makeschin, F. In an Ecuadorian pasture soil the growth of *Setaria sphacelata*, but not of soil microorganisms, is co-limited by N and P. *Appl. Soil Ecol.* 62, 103–114 (2012).
88. Johnson, N. C., Wilson, G. W. T., Wilson, J. A., Miller, R. M. & Bowker, M. A. Mycorrhizal phenotypes and the Law of the Minimum. *New Phytol.* 205, 1473–1484 (2015).
89. Barger, N. N., D'Antonio, C. M., Ghneim, T., Brink, K. & Cuevas, E. Nutrient limitation to primary productivity in a secondary savanna in Venezuela. *Biotropica* 34, 493 (2002).
90. Soudzilovskaia, N. A., Onipchenko, V. G., Cornelissen, J. H. C. & Aerts, R. Biomass production, N/P ratio and nutrient limitation in a Caucasian alpine tundra plant community. *J. Veg. Sci.* 16, 399–406 (2005).
91. Gill, R. A. et al. Linking community and ecosystem development on Mount St Helens. *Oecologia* 148, 312–324 (2006).
92. Craine, J. M., Morrow, C. & Stock, W. D. Nutrient concentration ratios and co-limitation in South African grasslands. *New Phytol.* 179, 829–836 (2008).
93. von Oheimb, G. et al. N/P ratio and the nature of nutrient limitation in *Calluna*-dominated heathlands. *Ecosystems* 13, 317–327 (2010).
94. Iversen, C. M., Bridgham, S. D. & Kellogg, L. E. Scaling plant nitrogen use and uptake efficiencies in response to nutrient addition in peatlands. *Ecology* 91, 693–707 (2010).
95. Laliberté, E. et al. Experimental assessment of nutrient limitation along a 2-million-year dune chronosequence in the south-western Australia biodiversity hotspot. *J. Ecol.* 100, 631–642 (2012).
96. Onipchenko, V. G. et al. Alpine plant functional group responses to fertiliser addition depend on abiotic regime and community composition. *Plant Soil* 357, 103–115 (2012).
97. Fisher, J. B. et al. Nutrient limitation in rainforests and cloud forests along a 3,000-m elevation gradient in the Peruvian Andes. *Oecologia*. 172, 889–902 (2013).
98. Cusell, C., Kooijman, A. & Lamers, L. P. M. Nitrogen or phosphorus limitation in rich fens? Edaphic differences explain contrasting results in vegetation development after fertilization. *Plant Soil* 384, 153–168 (2014).
99. Zhan, S., Wang, Y., Zhu, Z., Li, W. & Bai, Y. Nitrogen enrichment alters plant N/P stoichiometry and intensifies phosphorus limitation in a steppe ecosystem. *Environ. Exp. Bot.* 134, 21–32 (2017).

100. Tischer, A. et al. Above- and below-ground linkages of a nitrogen and phosphorus co-limited tropical mountain pasture system—responses to nutrient enrichment. *Plant Soil* 391, 333–352 (2015).
101. He, M. & Dijkstra, F. A. Phosphorus addition enhances loss of nitrogen in a phosphorus-poor soil. *Soil. Biol. Biochem.* 82, 99–106 (2015).
102. Chen, F. -S. et al. Nitrogen and phosphorus additions alter nutrient dynamics but not resorption efficiencies of Chinese fr leaves and twigs differing in age. *Tree. Physiol.* 35, 1106–1117 (2015).
103. Alvarez-Clares, S. & Mack, M. C. Do foliar, litter, and root nitrogen and phosphorus concentrations reflect nutrient limitation in a lowland tropical wet forest? *PLoS ONE* 10, e0123796 (2015).
104. Homeier, J. et al. Tropical Andean forests are highly susceptible to nutrient inputs—rapid effects of experimental N and P addition to an Ecuadorian montane forest. *PLoS ONE* 7, e47128 (2012).
105. Dai, X., Ouyang, Z., Li, Y. & Wang, H. Variation in yield gap induced by nitrogen, phosphorus and potassium fertilizer in North China Plain. *PLoS ONE* 8, e82147 (2013).
106. R Development Core Team, R. & R Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 1, 409 (2014).
107. Poisot, T. Te digitize package: extracting numerical data from scatterplots. *R J.* 3, 25–26 (2011).
108. Maherali, H., Oberle, B., Stevens, P. F., Cornwell, W. K. & McGlenn, D. J. Mutualism persistence and abandonment during the evolution of the mycorrhizal symbiosis. *Am. Nat.* 188, E113–E125 (2016).
109. Viechtbauer, W. Conducting meta-analyses in R with the metafor. *J. Stat. Sofw.* 36, 1–48 (2010).
110. Del Re, A. C. & Hoyt, W. T. MAd: meta-analysis with mean differences v.0.8-2 (CRAN, 2014); <http://cran.r-project.org/web/packages/MAd>

Acknowledgements

This study was supported by the International Program CryoCARB (MSM 7E10073—CryoCARB, Austrian Science Fund (FWF): I370-B17, German Federal Ministry of Education and Research (03F0616A)), project no. GA17-15229S and the SoWa Research Infrastructure funded by MEYS CZ grants LM2015075 and EF16_013/0001782—SoWa Ecosystems Research. S.M. acknowledges support from the Swedish Research Councils, Formas (2015-468) and VR (2016-04146) and the Bolin Centre for Climate Research. J.B., T.U. and H.S. were also supported by Czech Science Foundation project no. 16-18453 S. G.H. acknowledges the Joint Partnership Initiative project COUP and the Swedish Research Council grant no. E0689701 and the project CryoN funded by Academy of Finland (no. 132045). P.C. would also like to thank

TES program of the U.S. Department of Energy (DOE) Office of Science, Biological and Environmental Research (BER) for partial support at Pacific Northwest National Laboratory (PNNL). PNNL is operated by Battelle for DOE. X. Xu kindly shared his dataset on microbial biomass elemental composition. We also thank N. Hess and B. Bond-Lamberty for comments and language corrections to this manuscript.