

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

Supplementary Information

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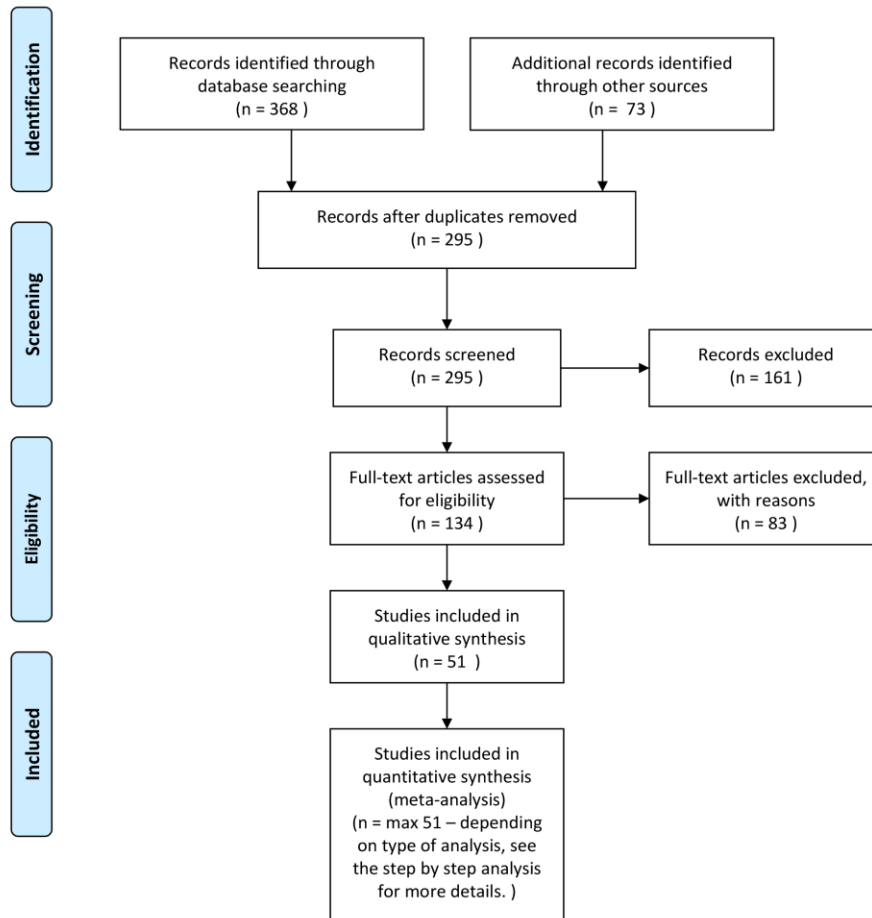
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1. Criteria for publications selection

To compare various predictions of proposed stoichiometric framework (see Fig. 1b of the main text) with published results, data from N-P factorial fertilization experiments, conducted at various local soil conditions, were collected. Based on our framework, the response of primary production to nitrogen (N) or phosphorus (P) fertilization is expected to depend on soil N to P ratio ($N:P_{SOIL}$), plant demand for N and P (defined as plant N to P critical ratio $N:P_{CR}^{plant}$), the demand of soil microbial community for N and P (defined as microbial N to P critical ratio $N:P_{CR}^{mic}$), the degree of competition or cooperation between plants and soil microbial communities and the availability of organic carbon (C) in soil. All these pieces of information were available only in one study, requiring us to establish a gap-filling procedure that allows using available information while avoiding spurious biases. Therefore, to obtain sufficiently robust dataset against which we can compare the framework predictions, we included all factorial fertilization experiments that report soil N and P concentrations and plant species composition in experimental plots. We assumed that $N:P_{CR}^{plant}$ and $N:P_{CR}^{mic}$ can be estimated as a function of $N:P_{SOIL}$, and that the degree of competition or cooperation of plants with the soil microbial community can be inferred from plant species composition (Brundrett 2009; Maherali et al. 2016). However, many methods are used to measure concentration of various forms of soil N and P. We considered studies reporting N and P in pools that are conceptually comparable (e.g., inorganic pool or total organic pool) to calculate a meaningful soil N to P ratio. A preliminary list of N-P factorial fertilization studies was extracted from the studies of LeBauer and Treseder (2008) and Harpole and co-authors (2011). 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. Based on these criteria, we identified 51 eligible studies (Supplementary Fig. 1).



PRISMA 2009 Flow Diagram



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit www.prisma-statement.org.

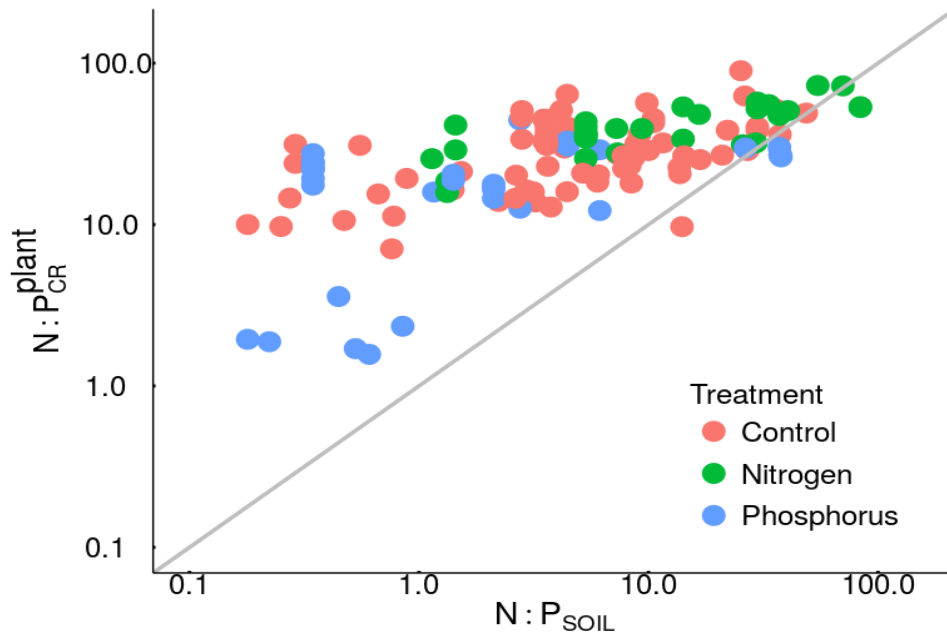
Supplementary Fig. 1: Prisma flow diagram of the publications selection procedure.

Relevant data were directly extracted from published studies using the program R and the package digitize (Poisot 2011). The dataset can be found on http://kbe.prf.jcu.cz/en/metaanalysis_data.

2. Gap filling

2.1. Plant critical N to P ratio ($N:P_{CR}^{plant}$)

The plant critical N to P ratio varies widely across studies. It has been suggested that plants adjust their demand according to changing stoichiometry of resources ($N:P_{SOIL}$) in order to avoid N or P limitation (Elser et al. 2007; Harpole et al. 2011). Therefore, a relationship between $N:P_{SOIL}$ and $N:P_{CR}^{plant}$ is expected. This relationship, can be used to calculate $N:P_{CR}^{plant}$ in studies in which $N:P_{SOIL}$ is reported but $N:P_{CR}^{plant}$ is not. To explore the relationship between $N:P_{SOIL}$ and $N:P_{CR}^{plant}$, data collected within this study are used. Desired dataset is found in the third spreadsheet of “metaanalysis_data.xlsx” file.

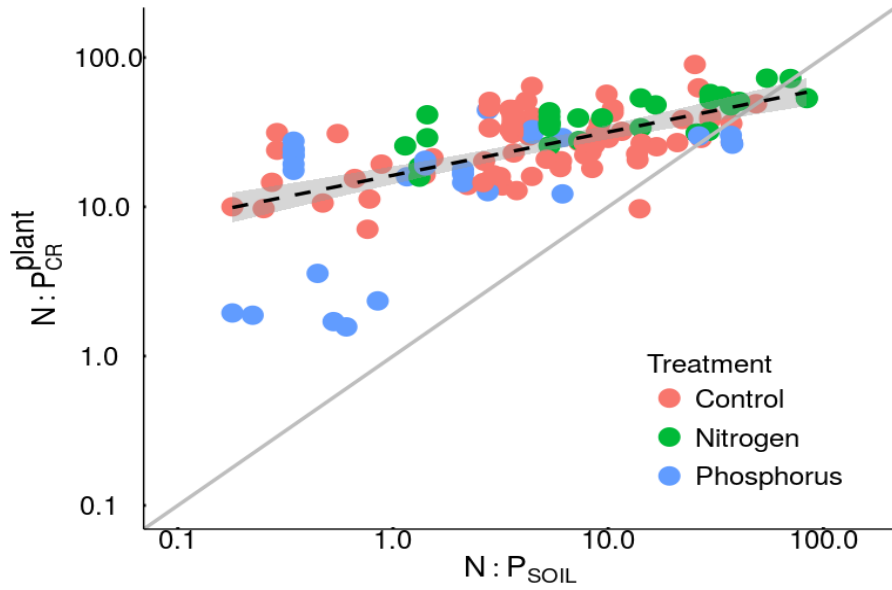


Supplementary Fig. 2: The relationship between soil N to P ratio ($N:P_{SOIL}$) and plant critical N to P ratio ($N:P_{CR}^{plant}$). Different colours designate different experimental treatments - no fertilization ($n=76$), nitrogen fertilization ($n=29$) and phosphorus fertilization ($n=29$). The grey line represents the 1:1 relationship. Note that both axes have logarithmic scales.

A visual inspection of the data suggests a power-law relationship between $N:P_{SOIL}$ and plant $N:P_{CR}^{plant}$; that is a linear relationship in a log-log plot. $N:P_{CR}^{plant}$ scales less-than-isometrically with $N:P_{SOIL}$, exhibiting a mildly non-homeostatic response to changes in soil resource N:P ratio.

Table S2: Results of linear regression between $\log_{10}(N : P_{SOIL})$ and $\log_{10}(N : P_{CR}^{plant})$. Mean value (Estimate) and corresponding standard errors (SE) of intercept and slope estimates are shown together adjusted R^2 of linear model.

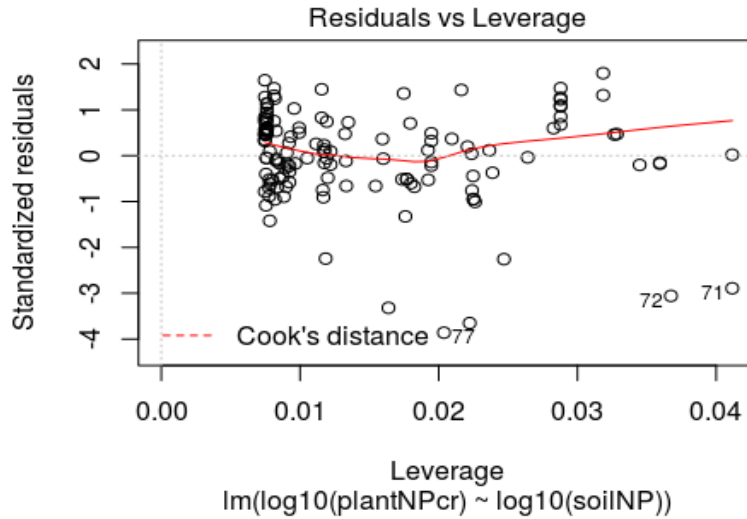
	Estimate	SE	p
Intercept	1.21	0.03	<0.001
Slope	0.29	0.03	<0.001
R^2			0.



Supplementary Fig. 3: The relationship between soil N to P ratio ($N:P_{SOIL}$) and plant critical N to P ratio ($N:P_{CR}^{plant}$). Different colours designate different experimental treatments - no fertilization ($n=76$), nitrogen fertilization ($n=29$) and phosphorus fertilization ($n=29$). The grey line represents the 1:1 relationship. The black dashed line represents linear regression and the grey area around the line represents the 95% confidence interval. Note that both axes have logarithmic scales.

On the logarithmic scale, both coefficients of the linear regression are highly significant (Supplementary Tab. 2). The intercept is 1.21 (± 0.03 SE) and the slope is 0.29 (± 0.03 SE), confirming the less-than-isometric scaling. Supplementary Fig. 3 suggests that six data points could be classified as outliers. These values are measured $N:P_{CR}^{plant}$ after P

fertilization. The effect of these values (observations 70 - 72 and 76 - 78) on the estimate of linear regression coefficients can be studied by Cook's distance plot.



Supplementary Fig. 4: Diagnostic plot of linear regression between $\log_{10}(N : P_{SOIL})$ and $\log_{10}(N : P_{CR}^{plant})$. Figure shows the standardized residuals plotted against the leverage of each observation.

Supplementary Fig. 4 shows no substantial effect of outlier values on the estimated linear regression coefficients. This can be verified by fitting the linear regression without outlier values.

Table S3: Results of linear regression between $\log_{10}(N : P_{SOIL})$ and $\log_{10}(N : P_{CR}^{plant})$ without outlier values (observations 70 - 72 and 76 - 78). Mean values (Estimate) and corresponding standard errors (SE) of intercept and slope estimates are shown together with adjusted R^2 of linear model.

	Estimate	SE	p
Intercept	1.32	0.02	<0.001
Slope	0.19	0.02	<0.001
R^2			0.32

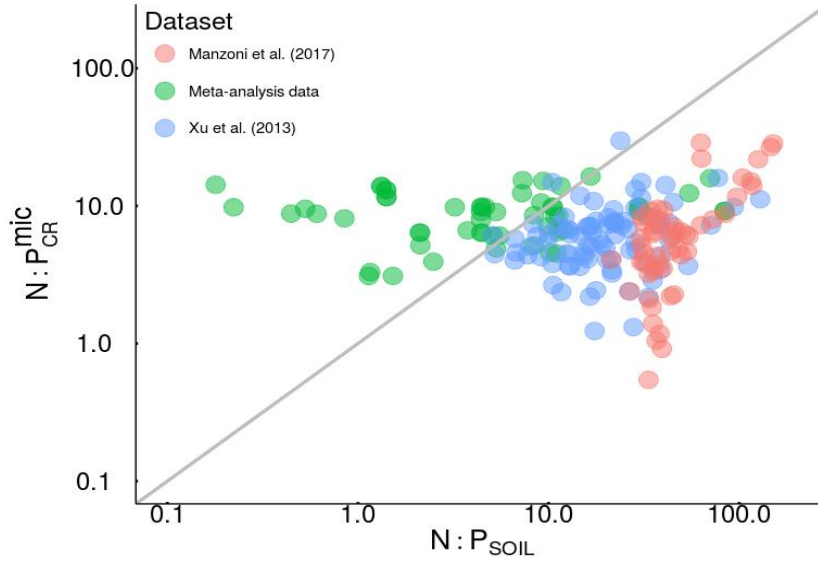
Table S4: Akaike Information Criterion (AIC) based comparison of two linear regressions between $\log_{10}(N : P_{SOIL})$ and $\log_{10}(N : P_{CR}^{plant})$ (with and without outlier values - observations 70 - 72 and 76 - 78). AIC and the AIC difference between models (ΔAIC) are shown. The lower AIC suggests better statistical model for a given dataset.

	AIC	ΔAIC	p
without outliers	-81.5		
with outliers	12.0	93.5	<0.001

By doing so, intercept increases from 1.21 ($\pm 0.03 SE$) to 1.32 ($\pm 0.02 SE$) and the slope decreases from 0.3 ($\pm 0.03 SE$) to 0.2 ($\pm 0.02 SE$). The differences in coefficients estimates are small, however, in terms of the quality of statistical model for a given dataset, which is characterized by Akaike Information Criterion, the difference is substantial (Supplementary Tab. 4). Therefore, observations 70 - 72 and 76 - 78 will be considered as outliers and the following linear relationship: $\log_{10}(N : P_{CR}^{plant}) = 1.32 + 0.2 \times \log_{10}(N : P_{SOIL})$ was used to calculate $N : P_{CR}^{plant}$ when only the $N : P_{SOIL}$ was reported.

2.2. Soil microbial community critical N to P ratio ($N : P_{CR}^{mic}$)

Contrasting hypotheses have been proposed to explain the relationship (or lack of thereof) between $N : P_{CR}^{mic}$ and $N : P_{SOIL}$. Some studies found that $N : P_{CR}^{mic}$ depends on $N : P_{SOIL}$ (flexible stoichiometry hypothesis (e.g. Godwin and Cotner 2015)), whereas others did not find any correlation (homeostatic hypothesis (e.g. Makino et al. 2003)). To explore the relationship between $N : P_{SOIL}$ and $N : P_{CR}^{mic}$, data collected within this study and two large literature datasets collected by Manzoni et al. (2017) (litter and organic soils) and Xu et al. (2013) (mostly mineral soils) are used.



Supplementary Fig. 5: The relationship between soil N to P ratio ($N:P_{SOIL}$) and microbial critical N to P ratio ($N:P_{CR}^{mic}$). Different colours designate different datasets (Meta-analysis data: $n=58$; Xu et al. (2013): $n=86$; Manzoni et al. (2017): $n=55$). The grey line represents 1:1 relationship. Note that both axes have logarithmic scales.

A visual inspection of the data suggests that no relationship between $N:P_{CR}^{mic}$ and $N:P_{SOIL}$ occurs. To verify the absence of a relationship, linear regression in log-log plot is conducted.

Table S5: Results of linear regression between $\log_{10}(N:P_{SOIL})$ and $\log_{10}(N:P_{CR}^{mic})$. Mean values (Estimate) and corresponding standard errors (SE) of intercept and slope estimates are shown together with adjusted R^2 of linear model.

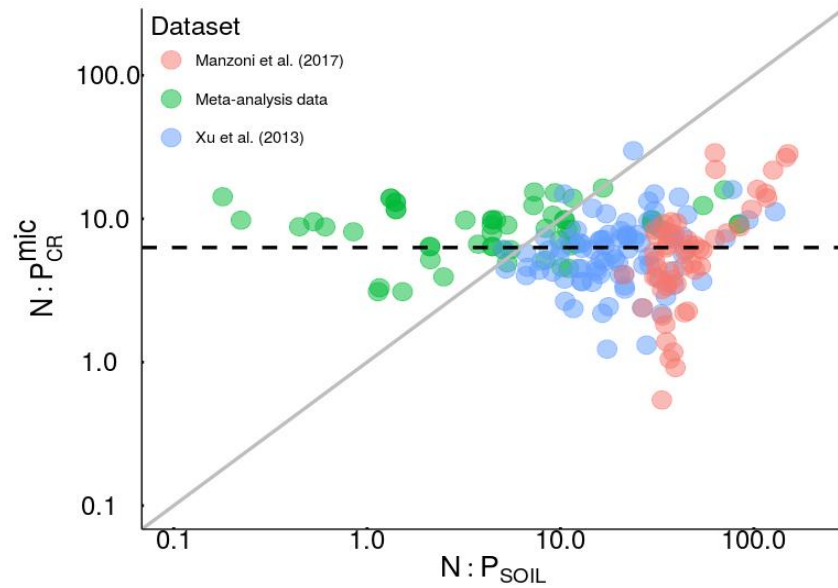
	Estimate	SE	p
Intercept	0.82	0.05	<0.001
Slope	-0.01	0.02	0.61
R^2			-0.004

The estimate of the linear regression slope is indeed not significantly different from zero (Supplementary Tab. 5). No relationship between $N:P_{CR}^{mic}$ and $N:P_{SOIL}$ can be assumed over the whole range of soil resource N:P ratios. The intercept estimate thus corresponds to the mean $N:P_{CR}^{mic}$.

Table S6: Intercept estimate for $\log_{10}(N : P_{CR}^{mic})$. Mean value (Estimate) and corresponding standard error (SE) is reported.

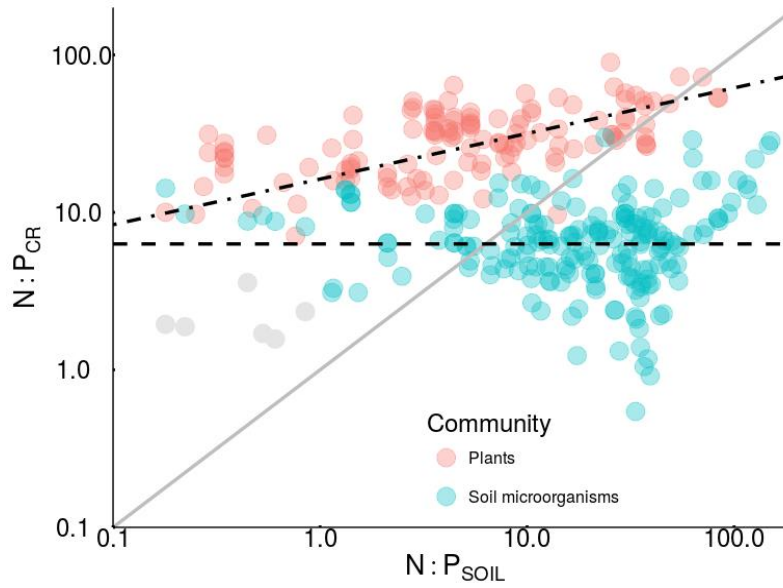
	Estimate	SE	p
Intercept	0.799	0.02	<0.001

After transformation from the logarithmic scale, the mean $N:P_{CR}^{mic}$ is $6.3 (\pm 1.0 SE)$. This value is independent of $N:P_{SOIL}$ and will be thus used as an estimate of $N:P_{CR}^{mic}$ in studies, which do not report it directly. By doing so, we are assuming homeostatic soil microbial community.



Supplementary Fig. 6: The relationship between soil N to P ratio ($N:P_{SOIL}$) and plant critical N to P ratio ($N:P_{CR}^{mic}$). Different colours designate different datasets (Meta-analysis data: $n=58$; Xu et al. (2013): $n=86$; Manzoni et al. (2017): $n=55$). The grey line represents 1:1 relationship. The black dashed line represents the mean $N:P_{CR}^{mic} = 6.3$. Note that both axes have logarithmic scales.

2.3. Plant and soil microbial community demand for N and P



Supplementary Fig. 7: The relationship between soil N to P ratio ($N:P_{SOIL}$) and critical N to P ratio ($N:P_{CR}$) of plants (red points, $n=134$) and soil microbial community (blue points, $n=199$). Grey line represents 1:1 relationship. Black dashed line represents the mean $N:P_{CR}^{mic} = 6.3$. Black dot-dashed line represents linear relationship between $N:P_{SOIL}$ and $N:P_{CR}^{plant}$. Grey points designate 6 outlier values of $N:P_{CR}^{plant}$. Note that both axis have a logarithmic scale.

Supplementary Fig. 7 combines supplementary figures 3 and 6. It shows that $N:P_{CR}^{plant}$ is generally higher than $N:P_{CR}^{mic}$ along wide gradient of $N:P_{SOIL}$. At low $N:P_{CR}^{plant}$, plants and soil microbial community are N-limited. As $N:P_{SOIL}$ increases above $6.3 (\pm 1.0 SE)$, soil microbial community becomes P-limited while plants are N-limited at the same time. Both, plants and soil microbial community is P-limited when $N:P_{SOIL}$ is higher than $42.4 (\pm 8.5 SE)$. It is important to emphasize that even if soil microbes exhibited a mild stoichiometric flexibility, our results would not change.

2.4. Microbial C-limitation

Our stoichiometric framework assumes that competition between plants and soil microbial community for nutrients occurs only when soil microorganisms are not limited by organic carbon (C). In C-limited conditions, free-living soil microorganisms mineralize nutrients, which are in excess to C and indirectly support nutrient limited plants. Therefore, it is necessary to determine if the soil microbial community is C limited at any given $N:P_{SOIL}$. We estimated the occurrence of soil microbial community C limitation by comparing measured total soil C:N and microbial community critical C to N ratio, which was calculated as the ratio of microbial biomass C:N and microbial C-use efficiency (CUE; Ågren et al. 2013; Bosatta and

Staaf 1982). Lacking more specific information, we assumed a CUE of 0.5 (Sinsabaugh et al. 2013) - a value reasonable for organic and mineral soils without strong nutrient limitation. In presence of strong nutrient limitation, CUE can be downregulated (Manzoni et al. 2017), so the critical C:N as defined here indicates the C:N threshold at which a C-limited microbial community starts immobilizing nutrients to fulfill their demands. If sustained immobilization continues, nutrient limitation will ensue.

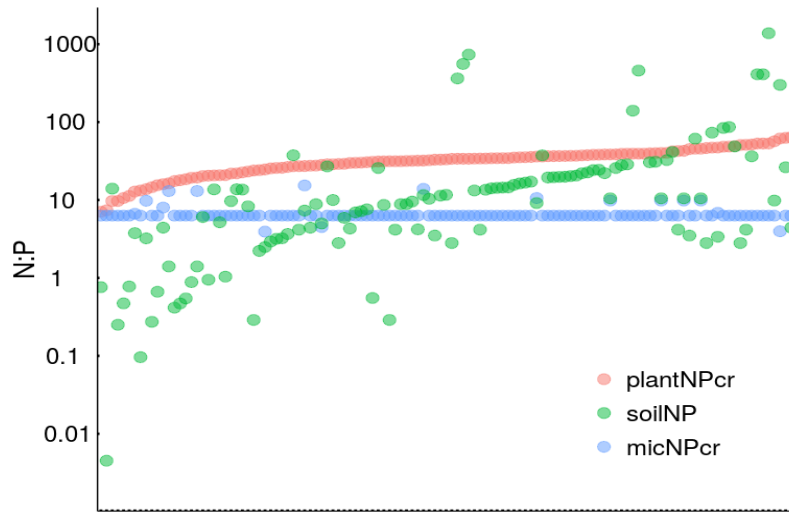
When microbial community C:N was not measured directly, we used the global mean value 6 (Xu, Thornton, and Post 2013). This was the case for all fertilization studies except two (Tab S1). When the total soil organic matter (SOM) C:N was higher than the critical C to N ratio of soil microbial community, or when glucose was added to the soil, soil conditions were considered C unlimited. Moreover, it was not possible to find total SOM C:N values for all studies (Supplementary Tab. 1). These studies are treated individually depending on the type of ecosystem or reported soil classification, and when the occurrence of C-limitation could not be unequivocally assessed, studies were treated as C-limited.

2.5. 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 as 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 and co-authors, 2016 and Brundrett, 2009). 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.

3. Meta-analysis

Meta-analysis dataset contains 264 missing values of $N: P_{CR}^{plant}$ and 396 missing values of $N: P_{CR}^{mic}$. Missing values are imputed as reported in sections 1.1. and 1.2..



Supplementary Fig. 8: Soil N to P ratio (soilNP; green points) and critical N to P ratio of plants (plantNPcr; red points) and soil microbial communities (micNPcr; blue points), as reported in the original publications or calculated for all studies and all experimental sites within studies. One experimental plot within one study is defined as one unique observation (for total of 124 unique observations) Data points are ordered according to increasing plantNPcr.

Table S7: Percentage of unique observations in which plants and soil microbial community are considered both N limited, P limited or, in which plants are considered N limited, but soil microbial community is considered P limited (N-P co-limitation). The definition of N or P limited soil conditions for plants and soil microbial community is illustrated in Fig. 1A of the main text. N or P limitation depends on the N to P ratio of soil resources and the N to P critical ratio of plants or soil microbial communities. While the N to P ratio of soil resources is measured in all studies, the N to P critical ratio of plants and soil microbial community is imputed for several observations. The imputation is connected with uncertainty. Because of this uncertainty, some observations can theoretically belong to more than one category of soil conditions.

Soil conditions	Mean	Mean + SE	Mean - SE
N limited	40.3 %	40.3 %	37.1 %
N-P co-limited	46.0 %	48.4 %	47.6 %
P limited	13.7 %	11.3 %	15.3 %

Supplementary Fig. 8 shows $N:P_{CR}^{plant}$, $N:P_{CR}^{mic}$ and $N:P_{SOIL}$ for each unique observation in the meta-analysis dataset. $N:P_{CR}^{plant}$ is higher than $N:P_{CR}^{mic}$ for all observations. Using the definition of plant and microbial N or P limitation reported in section 1.3., plants and soil microbial communities are considered N limited in 40.3% of all observations and P limited

in 13.7% of all observations. In 46.0% of all observations, plants are considered N limited and soil microbial community is considered P limited. However, there is uncertainty in respect to the definition of N and P limited conditions (calculated as a standard error of linear regression coefficients estimation, see section 1.3.). Due to this uncertainty, soil conditions cannot be exactly defined for some observations (Supplementary Tab. 7). C limited conditions for soil microbial community is identified in 11.2% of all observations.

3.1. Data preparation

The dataset includes a range of measured plant parameters, such as growth rate, and tissue nutrient concentration (all chosen parameters are listed in Supplementary Tab. 1). We use all these data types to test our hypotheses, following the rationale that several different parameters can serve as proxies for plant productivity, whose responses to nutrient additions we ultimately aim to predict. Many measured parameters related to plant productivity are correlated, while others are negatively correlated with productivity and are not useful proxies. Including these negatively correlated parameters in the meta-analysis is not meaningful, because their responses to nutrient addition are not expected to represent well the productivity responses. Therefore, we selected only those parameters that are related to productivity. Plants are considered N limited in the majority of observations (Supplementary Tab. 7). It is reasonable to expect that plant biomass, growth rate, and whole plant or shoot N concentration are positively correlated under N limited conditions. However, there is no reason to expect that plant P concentration correlates to plant productivity. For example, we expect that plant biomass, growth rate and N concentrations decrease after P fertilization when soil conditions are considered N limited for plants and P limited for the soil microbial community. However, plant P concentration may increase as result of luxury P consumption, which is not related to plant production. For this reason, all parameters connected to plant P nutrition are removed. By doing so, the number of observations in our dataset was not reduced.

Different studies use different methods to determine soil N and P concentrations. Among 51 studies included in our meta-analysis, total, extractable, ion exchange resin or pore water N and P is measured. We conduct our analysis on two data sets. First data set includes all studies. The second data set includes only studies which report extractable, ion exchange resin or pore water N and P. Compared to total soil N and P, these forms are regarded as more reliable in terms of their biological availability. In the second data set, however, 18 studies are missing and thus, one proposed hypothesis cannot be tested. In following tables, we report statistical analysis conducted on the complete data set. Separate statistical analyses conducted on the second data set are reported in the source code to this material and summarized in the Supplementary Fig. 16.

To obtain a set of independent values, an aggregation procedure was performed, as explained in the following.

Effect size is calculated as log Response ratio (logRR) for each observation in the dataset. logRR's are further aggregated in order to obtain one logRR for each unique combination of

$N:P_{CR}^{plant}$, $N:P_{CR}^{mic}$, $N:P_{SOIL}$, type and dose of fertilizer and the duration of fertilization experiment (time from start of the experiment to measurement). The Borenstein, Hedges, Higgins and Rothstein procedure, assuming correlation 0.3, is used as an aggregation procedure. By an aggregation procedure, the original dataset with 492 partly correlated observations was reduced to a dataset containing 316 independent observations.

3.2. Preliminary statistical evaluation

Before statistical evaluation of the predictions of our stoichiometric framework, reasonable and less complex hypotheses are tested using the dataset. The intention is to provide a general overview of the correspondence of the dataset with established hypotheses and to document the extent of variability, which cannot be accounted for by a particular hypothesis. If not stated otherwise, the coefficients of linear mixed-effect models (specified for a given hypothesis) are presented in all the following tables. The linear mixed-effect models are formulated as:

$$\log(RR) = \beta_0 + \beta_{x_i} \quad i = 1, 2, 3, \dots, n$$

In this equation, $\log(RR)$ is the magnitude of response of primary production (the dependent variable), β_0 is the intercept and β_{x_i} is the slope, which is estimated for a given predictor x_i (independent variable). For example $\beta_{Absolute.Latitude}$ designates the slope of the primary production response (i.e., $\log(RR)$) to increasing absolute latitude. Several (n) predictors can be theoretically specified in this equation. Statistical significance of each coefficient can be tested by an omnibus test of parameters. Additionally, a linear combination of model coefficients can be tested against zero. Thus, the hypothesis formulated as $\beta_0 + \beta_{x_i} = 0$ is tested. In the context of our framework, as explained in following example.

Example:

Using the linear mixed-effect model, we want to answer two specific questions:

- 1. Is the response of primary production (expressed as $\log(RR)$) to N fertilization significantly different from zero?*
- 2. Is the response of primary production (expressed as $\log(RR)$) to P fertilization significantly different from zero?*

In order to answer these questions, the following linear mixed-effect model can be used:

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

*in which the intercept β_0 defines the response of primary production ($\log(RR)$) to N fertilization and $\beta_{\text{Phosphorus}}$ defines the magnitude of additive effect of P fertilization. If the model coefficient $\beta_{\text{Phosphorus}}$ **is** statistically significant from zero, it does not mean that the response of primary production to P fertilization is significantly different from zero, but that the difference in the response of primary production to N and P fertilization is significant. If $\beta_{\text{Phosphorus}}$ **is not** statistically significant, the response of primary production to N and P fertilization is the same.*

To answer the first question, we test if the intercept β_0 is different from zero. To answer the second question, we test if the linear combination of both model coefficients is different from zero; that is if $\beta_0 + \beta_{\text{Phosphorus}} = 0$. For example, a linear combination of statistically significant coefficients $\beta_0 = 0.2$ and $\beta_{\text{Phosphorus}} = -0.4$ indicates that the response of primary production ($\log(RR)$) to P fertilization is significantly different from zero ($0.2 + (-0.4) = -0.2$) and specifically, it is lower than zero. Therefore, primary production increases after N fertilization ($\beta_0 = 0.2$) and decreases after P fertilization ($\beta_0 + \beta_{\text{Phosphorus}} = -0.2$).

*In a special case, the linear combination of statistically significant model coefficients does not need to be statistically different from zero. For example statistically significant coefficients $\beta_0 = 0.2$ and $\beta_{\text{Phosphorus}} = -0.2$ yield a linear combination equal to zero ($0.2 + (-0.2) = 0$). Based on this result we can conclude that the response of primary production ($\log(RR)$) to P fertilization **is not** significantly different from zero ($0.2 + (-0.2) = 0$). The interpretation of such a result is that the primary production increases after N fertilization and does not change after P fertilization.*

H1: Fertilization increases primary production.

Table S8: Results of the statistical test of H1. Size effect (logRR) estimate with corresponding standard error (SE) is shown together with AIC of the statistical model and the amount of residual variability (I^2).

	β_0	SE	p
	0.19	0.02	<0.001
AIC			460.7
$I^2(\%)$			99.4

As a first preliminary assessment of plant responses to fertilization, we tested if primary production is positively affected by nutrient additions (without specifying which nutrient). The statistical test provides a support for H1 (Supplementary Tab. 8), indicating that primary production generally and significantly increases after fertilization. On average, the primary production increases by a half ($10^{0.19} = 1.55$). I^2 quantifies the amount of residual variability, which is 99.4% of total variability. The response of primary production to fertilization is thus extremely variable and fertilizer addition alone is not a good predictor (despite being statistically significant). Therefore, other factors modify the response of primary production to fertilization are sought.

- **H2: The response of primary production to fertilization depends on the specific nutrient supplied with the fertilizer.**

Table S9: Results of the statistical test of H2. Linear mixed-effect model estimates of intercept (β_0 , nitrogen fertilizer) and $\beta_{\text{Phosphorus}}$ with corresponding standard errors (SE) are shown together with AIC of the statistical model and the amount of residual variability (I^2).

	Estimate	SE	p
β_0	0.24	0.03	<0.001
$\beta_{\text{Phosphorus}}$	-0.10	0.05	0.038
AIC			458.3
$I^2(\%)$			96.9

H2 is supported by the data (Supplementary Tab. 9). When N is supplied with the fertilizer, primary production increases by ($10^{0.23}$) 70%. When P is supplied with the fertilizer, primary production increases by only ($10^{0.23-0.09}$) 38%. The unexplained variability is still high (almost 97%) but slightly lower than in the previous more general case.

- **H3: The effect of fertilization depends on the dose of added fertilizer.**

Table S10: Results of the statistical test of H3. Linear mixed-effect model estimates of intercept (β_0 , nitrogen fertilizer), $\beta_{\text{Dose-Nitrogen}}$ and $\beta_{\text{Dose-Phosphorus}}$ with corresponding standard errors (SE) are shown together with AIC of the statistical model and the amount of residual variability (I^2).

	Estimate	SE	p
β_0	0.070	0.0357	0.051
$\beta_{\text{Dose-Nitrogen}}$	0.001	0.0003	0.003
$\beta_{\text{Dose-Phosphorus}}$	0.001	0.0003	<0.001
AIC			284.3
$I^2(\%)$			97.4

It has been suggested that higher doses of fertilizer can negatively affect primary production because of toxic effects. This dataset does not support this hypothesis (Supplementary Tab. 10). On the contrary, the more N or P is supplied with fertilizer, the higher the increase of primary production. This result shows that no toxicity occurs in the range of fertilizer additions used in the selected studies, but that increasing fertilizer dose has a positive effect.

- **H4: The effect of fertilization changes over time.**

Table S11: Results of the statistical test of H4. Linear mixed-effect model estimates of intercept (β_0 , nitrogen fertilizer), $\beta_{\text{Dose-Nitrogen}}$, $\beta_{\text{Dose-Phosphorus}}$ and β_{Duration} (experiment duration) with corresponding standard errors (SE) are shown together with AIC of the statistical model and the amount of residual variability (I^2).

	Estimate	SE	p
β_0	0.060	0.0370	0.104
$\beta_{\text{Dose-Nitrogen}}$	0.001	0.0003	0.003
$\beta_{\text{Dose-Phosphorus}}$	0.001	0.0003	<0.001
β_{Duration}	0.003	0.0027	0.253
AIC			285.7
$I^2(\%)$			97.4

It could be argued that fertilization effects may cumulate over time, as soil conditions slowly improve thanks to nutrient addition. However, the effect of fertilization does not change significantly over time (Supplementary Tab. 11).

It has been further suggested that the response of primary production to fertilization varies among ecosystems or along the latitudinal gradient (Elser et al. 2007). Both hypotheses are tested in the following.

- **H5: The effect of fertilization on primary production varies among ecosystems.**

Table S12: Results of the statistical test of H5. Linear mixed-effect model estimates of intercept (β_0 , nitrogen fertilizer) and β_{x_i} coefficients for phosphorus fertilizer and particular ecosystem are shown together with corresponding standard errors (SE). AIC of the statistical model and the amount of residual variability (I^2) is reported as well.

	Estimate	SE	p
β_0	0.291	0.1401	0.038
$\beta_{\text{Phosphorus}}$	-0.104	0.0481	0.030
$\beta_{\text{Crop_field}}$	0.057	0.2082	0.782
β_{Desert}	-0.156	0.1835	0.394
$\beta_{\text{Fir_plantation}}$	-0.174	0.1769	0.325
$\beta_{\text{Grassland}}$	-0.011	0.1492	0.943
$\beta_{\text{Mangrove_forest}}$	0.525	0.1950	0.007
β_{Savanna}	0.135	0.3119	0.664
β_{Steppe}	-0.174	0.1961	0.375
$\beta_{\text{Temperate_forest}}$	-0.238	0.2946	0.419
$\beta_{\text{Tropical_forest}}$	0.024	0.1517	0.874
β_{Tundra}	-0.175	0.1467	0.234
β_{Wetland}	-0.030	0.1462	0.836
AIC			449.1
$I^2(\%)$			95.7

- **H6: The effect of fertilization varies along the latitudinal gradient.**

Table S13: Results of the statistical test of H6. Linear mixed-effect model estimates of intercept (β_0 , nitrogen fertilizer), $\beta_{\text{Phosphorus}}$ and $\beta_{\text{Absolute-latitude}}$ with corresponding standard errors (SE) are shown together with AIC of the statistical model and the amount of residual variability (I^2).

	Estimate	SE	p
β_0	0.373	0.0632	<0.001
$\beta_{\text{Phosphorus}}$	-0.094	0.0476	0.049
$\beta_{\text{Absolute-latitude}}$	-0.003	0.0013	0.011
AIC			452.6
$I^2(\%)$			96.2

Hypotheses H5 and H6 are complementary to some extent. H5 can be rejected because primary production responds similarly to fertilization across different ecosystems. Mangrove forests in our dataset represent an exception, as primary production increases strongly after fertilization (Supplementary Tab. 12; $10^{(0.291+0.525)} = 6.5$). H6, on the other hand, is supported by data (Supplementary Tab. 13). Primary production responds more strongly to fertilization in low latitude areas, consistent with the generally accepted hypothesis that tropical areas tend to be relatively nutrient poor. Despite this significant effect, the fraction of explained variance increases marginally, suggesting that much variation is not due to fertilization per se or location (latitude).

3.3. Statistical evaluation of the stoichiometric framework predictions

The proposed stoichiometric framework generates several hypotheses, which can be directly tested. The hypotheses are visualized in Fig. 2 of the main text and listed in supplementary table 14.

Table S14: Testable hypothesis (N-H1 - C-H1) generated by the stoichiometric framework. The framework is explained in the main text and visualized in Fig. 1B. As long as the soil microbial community is not limited by organic carbon (Carbon unlimited), the response of primary production (calculated as $\log(RR)$; see section 1.2.) to nitrogen or phosphorus fertilization (Fertilizer) is expected to depend on soil N to P ratio (see Tab. 6 for definition), plant and microbial demand for N and P (see sections 2.1. - 2.3.) and the relationship between plants and soil microbial communities (competitive or cooperative). Cooperative soil microbial communities are not regarded as carbon limited because organic carbon is provided by plants. Competitive soil microbial communities can, however, be carbon limited at any time. Under carbon limitation, competitive soil microbial communities are expected to mineralize nutrients in excess.

Nutrient limitation	Fertilizer	Plant-microbe relationship	Hypothesis	$\log(RR)$
Carbon unlimited				
N limited plants and microorganisms	Nitrogen	competitive or cooperative	N-H1	> 0
	Phosphorus	competitive	N-H2	< 0
N limited plants, P limited microorganisms		cooperative	N-H3	= 0
	Nitrogen	competitive or cooperative	NP-H1	> 0
	Phosphorus	competitive	NP-H2	< 0
P limited plants and microorganisms		cooperative	NP-H3	> 0
	Nitrogen	competitive	P-H1	< 0
		cooperative	P-H2	= 0
	Phosphorus	competitive or cooperative	P-H3	> 0
Carbon limited				
Any condition	Any condition	competitive	C-H1	> 0

Ten specific hypotheses, which can be directly tested, are generated and tested in following. The dose of fertilizer and the duration of fertilization experiment were identified as important predictors of primary production response to fertilization (see section 2.2.), thus, their contribution is tested as well. All observations identified as influential are not removed from the dataset except of study of Cusell et al. (2014) (see section 3.3.2).

3.3.1. N limited soil conditions

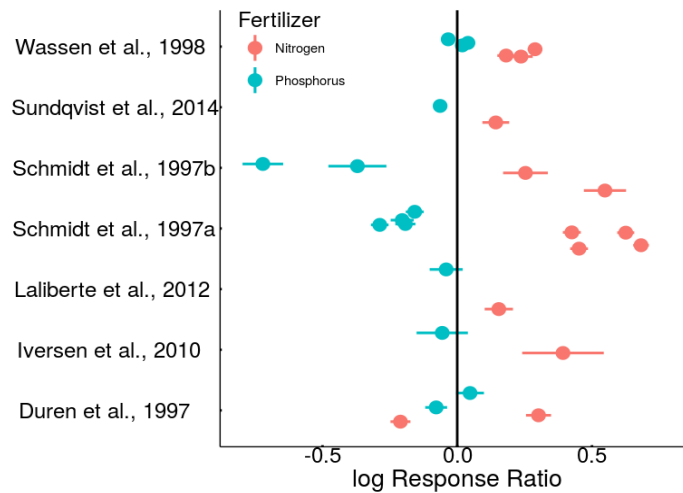
Competitive plant-microbe relationship (N-H1 and N-H2)

Table S15: Results the of statistical test of hypotheses N-H1 and N-H2. Size effect ($\log(RR)$) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{Phosphorus}$) additions with corresponding standard errors (SE) are shown together with AIC scores and the amount of residual variability (I^2). Both model coefficients are statistically significant (data not shown). The statistical model is specified for C unlimited conditions of the microbial community and plant-microbe competitive relationship. The effects of dose of fertilizer and duration of the fertilization experiment are not statistically significant, and therefore they are not reported.

	Estimate	SE	p
Nitrogen	0.30	0.06	<0.001 [*]
Phosphorus	-0.11	0.06	0.054 [†]
AIC			-0.29
$I^2(\%)$			79.57

^{*} The intercept (β_0) of mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and $\beta_{Phosphorus}$ is tested against zero using the Wald-Type test.



Supplementary Fig. 9: Mean response of primary production (calculated as log response ratio) to N (red points) and P (blue points) fertilization. Error bars represent corresponding standard errors. Soil conditions of all observations in the figure were identified as **N limited** (see Tab. A6 for definition) and **C unlimited**. The dominant type of plant-microbe relationship was identified as **competitive**. Observations are grouped according to the studies in which they are reported. The vertical line indicates log response ratio equal to zero (i.e., no response of primary production to fertilization).

Hypotheses N-H1 and N-H2 are supported by the data (Supplementary Tab. 15). Under C unlimited conditions, N fertilization significantly increases primary production, while P fertilization significantly decreases primary production at $p = 0.05$. Almost all observations support the predicted response (Supplementary Fig. 9). One observation reported in the study of Duren et al. (1997) is identified as an outlier (diagnostic plots are available in the source code to this document). When this observation is excluded from the dataset, the significance of the negative effect of P fertilization increases and the amount of residual variability decreases (results are available in the source code to this document).

Cooperative plant-microbe relationship (N-H1 and N-H3)

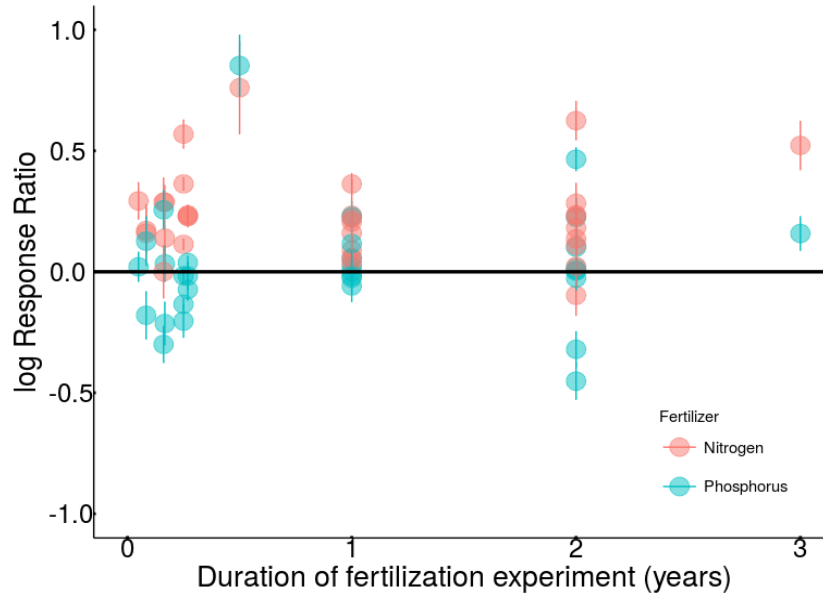
Table S16: Results of the statistical test of hypothesis N-H1 and N-H3. Size effect ($\log(RR)$) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{Phosphorus}$) additions and corresponding standard errors (SE) are shown together with AIC scores and the amount of residual variability (I^2). Significant effect of the duration of fertilization experiment ($\beta_{Duration}$) is reported as well. All coefficients of linear mixed-effect model are statistically significant (data not shown). The statistical model is specified for cooperative plant-microbe relationship.

	Estimate	SE	p
Nitrogen	0.193	0.025	<0.001 [*]
Phosphorus	0.000	0.025	0.997 [†]
$\beta_{Duration}$	0.003	0.001	<0.001 [*]
AIC			-38.70
$I^2(\%)$			71.47

^{*} The intercept (β_0) of mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and $\beta_{Phosphorus}$ is tested against zero using the Wald-Type test.

^{*} The model coefficient $\beta_{Duration}$ is tested against zero using the Wald-Type test.



Supplementary Fig. 10: Mean response of primary production (calculated as log Response ratio) to N (red points) and P (blue points) fertilization over time. Error bars represent corresponding standard errors. The horizontal line indicates log response ratio equal to zero (i.e., no response of primary production to fertilization).

Hypothesis N-H1 and N-H3 are supported by the data. The response of primary production to P fertilization is not significant, while N fertilization increases plant production (Supplementary Tab. 16). The duration of fertilization experiment slightly, but significantly, increases the response of primary production to fertilization (Supplementary Fig. 10). The effect of microbial carbon limitation on the response of primary production to fertilization was tested and found non-significant (data not shown). Two observations of the study of Watanabe et al. (2015) are identified as outliers (diagnostic plots are included in the source code to this document). When these observations are excluded from the dataset, duration of the fertilization experiment becomes non-significant and the residual variability decreases from 71.5 to 62.5 (results are available in the source code to this document). Hypothesis N-H1 is tested together with N-H2 and N-H3. As also stated above, under both competitive and cooperative plant-microbe relationship, N fertilization significantly increases primary production (Tabs. S15 and S16). Thus, hypothesis N-H1 is supported by data.

3.3.2. N-P co-limited soil conditions

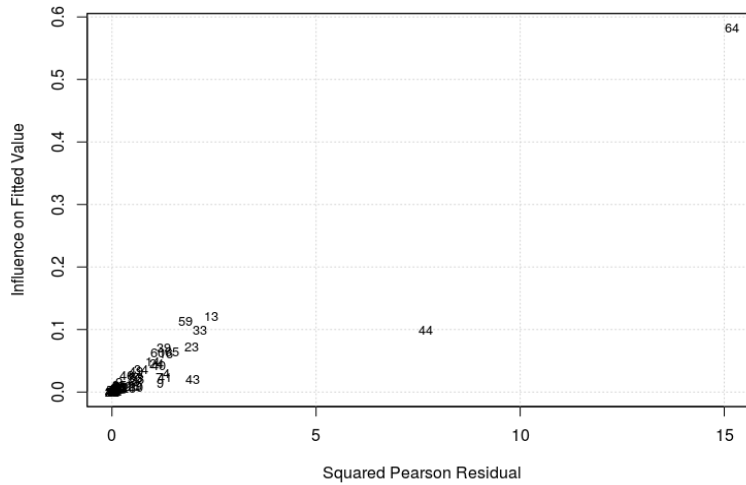
Competitive plant-microbe relationship (NP-H1 and NP-H2)

Table S17: Results of the statistical test of hypotheses NP-H1 and NP-H2. Size effect ($\log(RR)$) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{Phosphorus}$) additions with corresponding standard errors (SE) are shown together with AIC scores and the amount of residual variability (I^2). The statistical model is specified for C unlimited conditions of the microbial community and competitive plant-microbe relationship. One influential observation reported in study of Cusell et al. (2014) was excluded from the dataset (see Figs. S11 and S12). The effects of dose of fertilizer and the duration of the fertilization experiment are not statistically significant and therefore they are not reported.

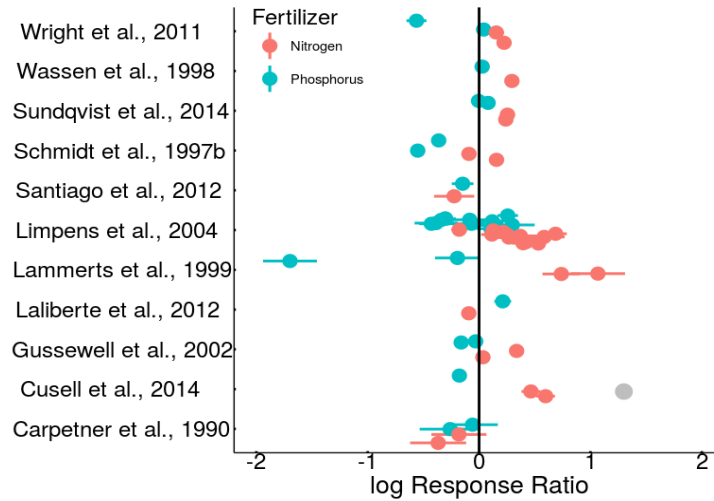
	Estimate	SE	p
Nitrogen	0.23	0.05	<0.001 [*]
Phosphorus	-0.11	0.05	0.034 [†]
AIC			27.45
$I^2(\%)$			53.49

^{*} The intercept (β_0) of mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and $\beta_{Phosphorus}$ is tested against zero using the Wald-Type test.



Supplementary Fig. 11: Baujat plot for the statistical model, which test the effect of fertilization on primary production under N-P co-limited, C unlimited soil conditions and competitive plant-microbe relationship. The plot shows the contribution of each observation to the overall model fit. Observation no. 64 is detected as a highly influential (see also Supplementary Fig. 12). This observation is reported in study of Cusell et al. (2014). Because $N:P_{SOIL}$ (48.7) is the same as $N:P_{CR}^{plant}$ (49.1) for this observation (plant N-P co-limited conditions), the observation is considered as an outlier and it is excluded from the dataset.



Supplementary Fig. 12: Mean response of primary production (calculated as log response ratio) to N (red points) and P (blue points) fertilization. Error bars represent corresponding standard errors. Soil conditions of all observations in the figure were identified as **N-P co-limited** (see Tab. A6 for definition) and **C unlimited**. The dominant type of plant-microbe relationship was identified as **competitive**. Observations are grouped according to the studies in which they are reported. The vertical line indicates a log response ratio equal to zero (i.e., no response of primary production to fertilization). Study of Cusell et al. (2014) is identified as an outlier (grey point; see Supplementary Fig. 11 for explanation).

Hypotheses NP-H1 and NP-H2 are supported by the data (Supplementary Tab. 17). However, the statistical test of hypothesis NP-H2 is significant only when one observation from the study of Cusell et al. (2014) is excluded from the dataset. This observation has great influence on the overall statistic (Figs. S11 and S12). Including this observation, the negative effect of P fertilization is still present but not statistically significant ($p = 0.269$), and the I^2 increases from 53.5% to 72.1%. This observation has practically undistinguishable $N: P_{SOIL}$ (48.7) and $N: P_{CR}^{plant}$ (49.1), suggesting that plants are N-P co-limited. Therefore, their response to N and P fertilization is expected to be positive.

Cooperative plant-microbe relationship (NP-H1 and NP-H3)

Table S18: Results of the statistical test of hypothesis NP-H3. Size effect (logRR) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{\text{Phosphorus}}$) additions and corresponding standard errors (SE) are shown together with AIC scores and the amount of residual variability (I^2). Only the intercept (β_0) of the linear mixed-effect model is statistically significant (data not shown). Therefore, alternative hypothesis (significant positive effect of fertilization* on primary production) is tested using the reduced model. The model is specified for cooperative plant-microbe relationship. The effects of dose of fertilizer and the duration of the fertilization experiment are not statistically significant and therefore, they are not reported.

	Estimate	SE	p
Nitrogen	0.170	0.054	0.002*
Phosphorus	0.239	0.056	<0.001 [†]
AIC			114.988
I^2			87.479
Alternative hypothesis			
Fertilization*	0.203	0.039	<0.001*
AIC			113.722
$I^2(\%)$			88.628

* The intercept (β_0) of mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and $\beta_{\text{Phosphorus}}$ is tested against zero using the Wald-Type test.

* Reduced model (excluded non-significant $\beta_{\text{Phosphorus}}$) is fitted.

Hypotheses NP-H1 and NP-H3 are supported by the data: the positive response of primary production to both N fertilization and P fertilization is significant and positive (Supplementary Tab. 18). The effect of microbial carbon limitation on the response of primary production to fertilization was tested and found non-significant (data not shown). Three observations are identified as outliers (diagnostic plots are available in the source code to this document). When these observations are excluded from the dataset, the hypothesis NP-H3 is still supported and I^2 decreases drastically - from 87.5% to 53.6% (results are available in the source code to this document). As in the case of N-limited plants and microorganisms, under both competitive and cooperative plant-microbe relationship, N fertilization significantly increases primary production (Tabs. S17 and S18). Thus, as also stated above, hypothesis NP-H1 is supported by the data.

3.3.3. P limited soil conditions

Competitive plant-microbe relationship (P-H1)

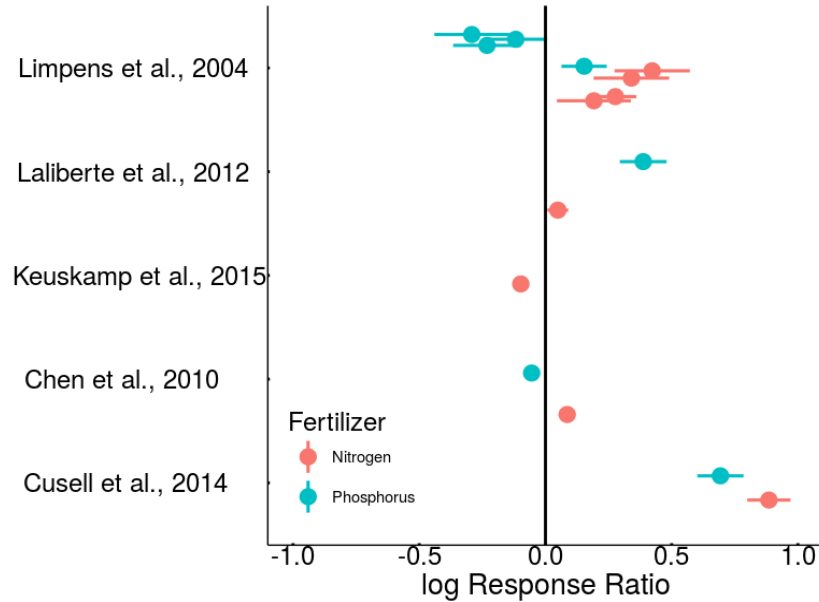
Table S19: Results of the statistical test of hypotheses P-H1. Size effect ($\log(RR)$) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{Phosphorus}$) additions with corresponding standard errors (SE) are shown together with AIC scores and the amount of residual variability (I^2). The statistical model is specified for C unlimited conditions of the microbial community and competitive plant-microbe relationship. Only the intercept (β_0) of the linear mixed-effect model is statistically significant (data not shown). Therefore, the alternative hypothesis (significant positive effect of fertilization* on primary production) is tested using the reduced model. The effect of dose of fertilizer and the duration of the fertilization experiment are not statistically significant and therefore, they are not reported.

	Estimate	SE	p
Nitrogen	0.24	0.15	0.100*
Phosphorus	0.24	0.15	0.094 [†]
AIC			20.32
I^2			92.74
Alternative hypothesis			
Fertilization*	0.24	0.10	0.015*
AIC			18.36
$I^2(\%)$			96.51

* The intercept (β_0) of mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and $\beta_{Phosphorus}$ is tested against zero using the Wald-Type test.

* Reduced model (excluded non-significant $\beta_{Phosphorus}$) is fitted.



Supplementary Fig. 13: Mean response of primary production (calculated as log Response ratio) to N (red points) and P (blue points) fertilization. Error bars represent corresponding standard errors. Soil conditions of all observations in the figure were identified as **P limited** (see Tab. A6 for definition) and **C unlimited**. The dominant type of plant-microbe relationship was identified as **competitive**. Observations are grouped according to studies, in which they are reported. Vertical line designate log Response ratio zero, which stands for no response of primary production to fertilization.

Hypothesis P-H1 is not supported by the data (Supplementary Tab. 19). No difference between the effect of N and P fertilization on the primary production exists. Both nutrients significantly increase primary production. One influential observation was found (diagnostic plots are available in the source code to this document), but it does not change the results of the tested hypothesis (results are available in the source code to this document).

Cooperative plant-microbe relationship (P-H2)

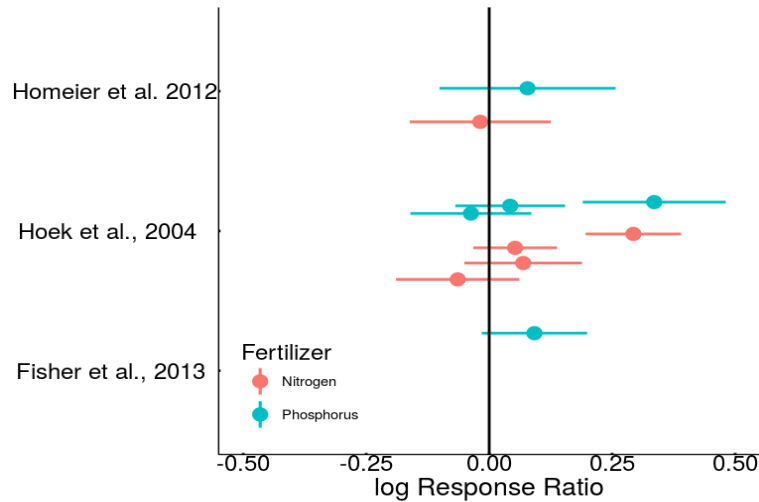
Table S20: Results of the statistical test of hypothesis P-H2. Size effects ($\log(RR)$) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{\text{Phosphorus}}$) additions and corresponding standard errors (SE) are shown together with AIC scores. The residual variability (I^2) was not possible to estimate, therefore, it is not reported. Only the intercept (β_0) of the linear mixed-effect model is statistically significant (data not shown). Therefore, the alternative hypothesis (significant positive effect of fertilization* on primary production) is tested using reduced model. The model is specified for cooperative plant-microbe relationship. The effects of dose of fertilizer and the duration of the fertilization experiment are not statistically significant and therefore they are not reported.

	Estimate	SE	p
Nitrogen	0.148	0.093	0.113*
Phosphorus	0.139	0.096	0.149†
AIC			1.547
$I^2(\%)$			
Alternative hypothesis			
Fertilization*	0.144	0.067	0.032*
AIC			-1.530
$I^2(\%)$			

* The intercept (β_0) of mixed-effect linear model is tested against zero using the Wald-Type test.

† The linear combination of model coefficients β_0 and $\beta_{\text{Phosphorus}}$ is tested against zero using the Wald-Type test.

* The reduced model (excluded non-significant $\beta_{\text{Phosphorus}}$) is fitted.



Supplementary Fig. 14: Mean response of primary production (calculated as log response ratio) to N (red points) and P (blue points) fertilization. Error bars represent corresponding standard errors. Soil conditions of all observations in the figure were identified as **P limited** (see Tab. A6 for definition). The dominant type of plant-microbe relationship was identified as **cooperative**. Observations are grouped according to the studies, in which they are reported. The vertical line indicates log response ratio equal to zero (i.e., no response of primary production to fertilization).

P-H2 is not supported by the data (Supplementary Tab. 20): primary production increases to the same extent after N and P fertilization. No influential observations are present in the dataset (diagnostic plots are available in the source code to this document). To summarize the results from Section 3.3., under both competitive and cooperative plant-microbe relationships, P fertilization significantly increases primary production (Tabs. S19 and S20) and thus, hypothesis P-H3 is supported.

3.4. Organic carbon limitation of soil microbial communities

Table S21: Results of statistical test of hypothesis C-H1. Size effect ($\log(RR)$) estimates for nitrogen (β_0) and phosphorus ($\beta_0 + \beta_{Phosphorus}$) additions and corresponding standard errors (SE) are shown together with AIC scores and the residual variability (I^2). Only the intercept (β_0) of the linear mixed-effect model is statistically significant (data not shown). Therefore, the alternative hypothesis (significant positive effect of fertilization[§] on primary production) is tested using the linear mixed-effect model. The hypothesis is tested for plant N limited and P limited conditions. The model is specified for carbon limited conditions of the microbial community and competitive plant-microbe relationship.

	Estimate	SE	p
Nitrogen	0.871	0.327	0.008*
Phosphorus	1.268	0.328	<0.001 [†]
AIC			121.130
I^2			99.172
Alternative hypothesis			
Fertilization _{N limitation}	0.635	0.306	0.04*
Fertilization _{P limitation}	0.889	0.445	<0.001 [‡]
AIC			118.027
I^2			99.274

* The intercept (β_0) of the mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and $\beta_{Phosphorus}$ is tested against zero using the Wald-Type test.

[‡] The linear combination of model coefficients β_0 and $\beta_{P limitation}$ is tested against zero using the Wald-Type test.

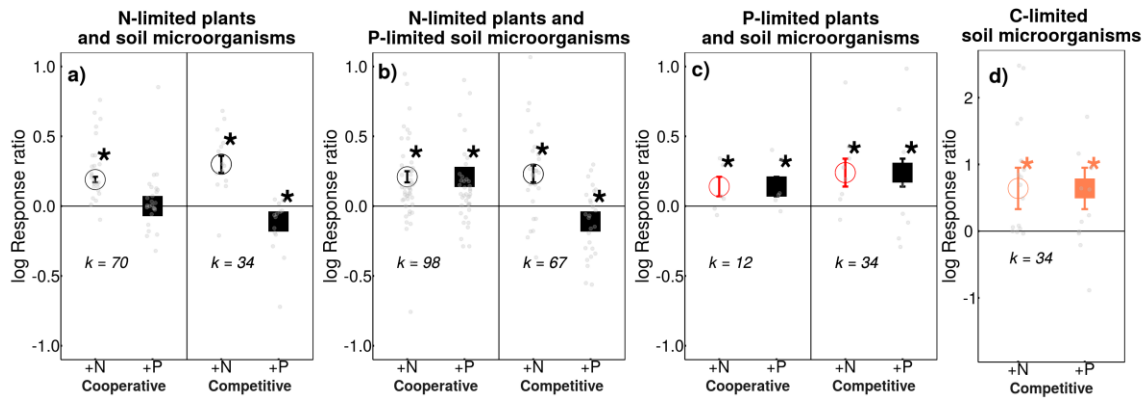
[§] The alternative model $\log(RR) = \beta_0 + \beta_{P limitation}$ is fitted, where β_0 represents $\log(RR)$ under plant N limited soil conditions and $\beta_{P limitation}$ represents additive $\log(RR)$ under plant P limited conditions.

Hypothesis C-H1 is supported by the data (Supplementary Tab. 21). No significant difference between N and P fertilization was identified, thus the response of primary production to fertilization is positive under C limited conditions. Interestingly, a significant difference was found between the response ratios of N limited and P limited plants. Under C limited conditions for soil microbial community, the response of primary production to N and P fertilization is higher under plant P limited conditions in comparison to plant N limited conditions.

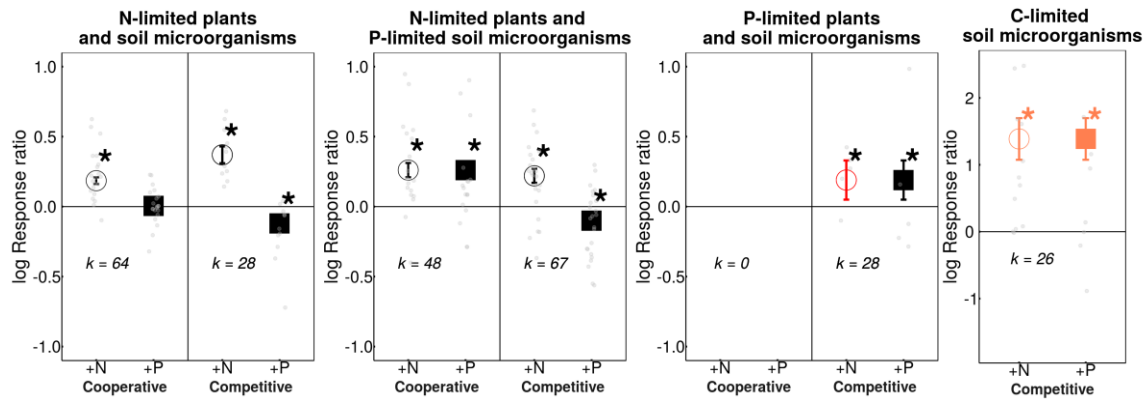
3.5. Summary of statistical evaluation of framework predictions

Results of statistical testing of the stoichiometric framework predictions are summarized in figure A15. All predictions except two are supported by the data. The only two not supported are hypotheses P-H1 and P-H2. Under P limited conditions, both P and N fertilization increases primary production.

The results of statistical testing of the stoichiometric framework predictions using only the studies which measured extractable, ion exchange resin and pore water N and P are summarized in figure S16. The same conclusions are reached with one exception. Hypothesis P-H2 could not be tested because of the lack of available data



Supplementary Fig. 15: Log response ratios of published data related to primary productivity to N (open circles) or P (closed squares) fertilization (see Methods section of the main text). The log Response ratios were estimated by linear mixed-effect models for 6 different categories. These categories distinguish between initial nutrient soil conditions under which plants and soil microorganisms are both N limited ($N: P_{SOIL} < \text{plant and microbial } N: P_{CR}$), plants are N limited while soil microorganisms are P limited ($N: P_{SOIL} > N: P_{CR}^{mic}$ and $N: P_{SOIL} < N: P_{CR}^{plant}$) and plants and soil microorganism are both P limited ($N: P_{SOIL} > \text{plant and microbial } N: P_{CR}$). Under each of these different soil conditions, plants and soil microorganisms may (“cooperative”) or may not (“competitive”) create symbiotic associations with plants. Mean values with standard errors are plotted. The horizontal line indicates a log response ratio of 0, which denotes no change (negative/positive log response ratios indicate a net decrease/increase in productivity after fertilization). The number of observations (k) are indicated for each category. All estimates marked by * are significantly different from zero at $p \leq 0.05$. Red points indicate no correspondence between framework predictions and the statistical results. Orange symbols represent the response ratios when competitive soil microorganisms are C-limited. Grey dots represent the original response ratios.



Supplementary Fig. 16: The summary of statistical analyses conducted on the subset of studies, which measured extractable, ion exchange resin or pore water N and P as directly available forms. For more details see Supplementary Fig. 15 and the section 3.1.

3.6. Statistical evaluation of response groups

All hypotheses listed in Supplementary table 14 predict one of the three possible responses of primary production to N or P fertilization - positive response, negative response or no response. Therefore, it is possible to define more general hypotheses by grouping data based solely on the sign of the response. These hypotheses can be directly tested across all observations in the dataset (Supplementary Tab. 23).

Table S22: Three general hypotheses generated by the conceptual framework. For the explanation of particular hypotheses see Tab. S14. According to figure S15, two out of ten hypotheses are not supported by data.

	Hypotheses	logRR
Positive response	N-H1, NP-H1, NP-H3, P-H3, C-H1	>0
Negative response	N-H2, NP-H2, P-H1*	<0
No response	N-H3, P-H2*	=0

* These hypotheses are not supported by data (see Fig. S15).

To perform this more general test, all observations in the dataset are grouped according to the expected response of primary production to either N or P fertilization. The effect of one

composite categorical predictor with three different levels (Positive, Negative and Zero) is tested using linear mixed-effect model.

Table S23: Results of the linear mixed-effect model that tests the significance of three composite hypotheses obtained by grouping all predictions of **positive**, **negative** or **no** response of primary production (calculated as log response ratio) to fertilization. Model coefficients, corresponding standard errors, AIC scores and amount of residual variability (I^2) are reported. Using the model, three hypotheses are tested^{*,†}. Results are generated for the framework predictions **without** accounting for the possible effect of nitrogen as a mediator of extracellular enzyme activity.

	Estimate	SE	p
Linear mixed-effect model			
β_0	-0.07	0.06	0.208
β_{No}	0.10	0.08	0.202
$\beta_{Positive}$	0.35	0.06	<0.001
AIC			426.4
$I^2(\%)$			96.1
Tested hypotheses			
Negative response	-0.07	0.06	0.208 [*]
No response	0.03	0.06	0.582 [†]
Positive response	0.28	0.03	<0.001 [‡]

^{*} The intercept (β_0 , **negative response**) of mixed-effect linear model is tested against zero using the Wald-Type test.

[†] The linear combination of model coefficients β_0 and β_{No} (**no response**) is tested against zero using the Wald-Type test.

[‡] The linear combination of model coefficients β_0 and $\beta_{Positive}$ (**positive response**) is tested against zero using the Wald-Type test.

Table S24: Results of the linear mixed-effect model that tests the significance of three composite hypotheses obtained by grouping all predictions of **positive**, **negative** or **no** response of primary production (calculated as log response ratio) to fertilization. Model coefficients, corresponding standard errors, AIC scores and amount of residual variability (I^2) are reported. Using the model, three hypotheses are tested⁺⁺. Results are generated for the framework predictions accounting for the possible effect of nitrogen as a mediator of extracellular enzyme activity.

	Estimate	SE	p
Linear mixed-effect model			
β_0	-0.13	0.06	0.031
β_{No}	0.15	0.09	0.078
$\beta_{Positive}$	0.41	0.07	<0.001
AIC			421.1
$I^2(\%)$			96.1
Tested hypotheses			
Negative response	-0.13	0.06	0.031 [*]
No response	0.02	0.06	0.748 [†]
Positive response	0.28	0.03	<0.001 [‡]

^{*} The intercept (β_0 , **negative response**) of mixed-effect linear model is tested against zero by Wald-Type test.

[†] The linear combination of model coefficients β_0 and β_{No} (**no response**) is tested against zero using the Wald-Type test.

[‡] The linear combination of model coefficients β_0 and $\beta_{Positive}$ (**positive response**) is tested against zero using the Wald-Type test.

The data do not support all the composite hypotheses stemming from the stoichiometric framework (Supplementary Tab. 23), because the expected negative responses of primary production are in fact not statistically different from zero. The model coefficient β_{No} is not significant either, because hypotheses P-H1 and P-H2 are not supported by the data; i.e., N addition to P-limited plants in competitive and cooperative systems improves productivity instead of having a negative and neutral effect, respectively. As explained in the main text, we argue that N addition can improve P acquisition by fostering extracellular enzyme production, which in turn allows access to organically bound P to plants. When the predictions of this extended framework are tested, data offer strong support (Supplementary Tab. 24). Two influential observations are found in the whole dataset (data not shown). When these observations are excluded from the dataset, the significance of the linear mixed-effect model coefficient estimates increases and the residual variability

decreases from 96.1 to 92.7. We emphasize that the proposed stoichiometric framework is meant to predict the sign of the response of primary productivity to fertilization, and not the extent of the response. Therefore, having coefficients that are significantly different from zero with signs consistent with the framework predictions is enough to support the framework. The high unexplained variance would require a process-understanding of the extent of vegetation responses that is beyond our scope here.

4. References

Ågren, G I, R Hyvonen, S L Berglund, and S E Hobbie. 2013. "Estimating the critical N:C from litter decomposition data and its relation to soil organic matter stoichiometry." *Soil Biology and Biochemistry* 67: 312–18. doi:<http://dx.doi.org/10.1016/j.soilbio.2013.09.010>.

Bosatta, Ernesto, and Håkan Staaf. 1982. "The Control of Nitrogen Turn-Over in Forest Litter." *Oikos* 39 (2): 143. doi:[10.2307/3544478](https://doi.org/10.2307/3544478).

Brundrett, Mark C. 2009. "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 and Soil* 320 (1-2): 37–77. doi:[10.1007/s11104-008-9877-9](https://doi.org/10.1007/s11104-008-9877-9).

Cusell, Casper, Annemieke Kooijman, and Leon P. M. Lamers. 2014. "Nitrogen or phosphorus limitation in rich fens? - Edaphic differences explain contrasting results in vegetation development after fertilization." *Plant and Soil* 384 (1-2). Springer International Publishing: 153–68. doi:[10.1007/s11104-014-2193-7](https://doi.org/10.1007/s11104-014-2193-7).

Elser, James J., Matthew E S Bracken, Elsa E. Cleland, Daniel S. Gruner, W. Stanley Harpole, Helmut Hillebrand, Jacqueline T. Ngai, Eric W. Seabloom, Jonathan B. Shurin, and Jennifer E. Smith. 2007. "Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems." *Ecology Letters* 10 (12). Blackwell Publishing Ltd: 1135–42. doi:[10.1111/j.1461-0248.2007.01113.x](https://doi.org/10.1111/j.1461-0248.2007.01113.x).

Godwin, Casey M, and James B Cotner. 2015. "Aquatic heterotrophic bacteria have highly flexible phosphorus content and biomass stoichiometry." *The ISME Journal* 9 (10). NATURE PUBLISHING GROUP, MACMILLAN BUILDING, 4 CRINAN ST, LONDON N1 9XW, ENGLAND: 2324–7. doi:[10.1038/ismej.2015.34](https://doi.org/10.1038/ismej.2015.34).

Harpole, W. Stanley, Jacqueline T. Ngai, Elsa E. Cleland, Eric W. Seabloom, Elizabeth T. Borer, Matthew E.S. Bracken, James J. Elser, et al. 2011. "Nutrient co-limitation of primary producer communities." *Ecology Letters* 14 (9). Blackwell Publishing Ltd: 852–62. doi:[10.1111/j.1461-0248.2011.01651.x](https://doi.org/10.1111/j.1461-0248.2011.01651.x).

LeBauer, David S, and Kathleen K Treseder. 2008. "Nitrogen limitation of net primary productivity in terrestrial ecosystems is globally distributed." *Ecology* 89 (2): 371–79. doi:[10.1890/06-2057.1](https://doi.org/10.1890/06-2057.1).

Maherali, Hafiz, Brad Oberle, Peter F Stevens, William K Cornwell, and Daniel J McGlinn. 2016. "Mutualism Persistence and Abandonment during the Evolution of the Mycorrhizal Symbiosis." *The American Naturalist* 188 (5). UNIV CHICAGO PRESS, 1427 E 60TH ST, CHICAGO, IL 60637-2954 USA: E113–E125. doi:[10.1086/688675](https://doi.org/10.1086/688675).

Makino, W, J B Cotner, R W Sterner, and J J Elser. 2003. "Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C : N : P stoichiometry." *Functional Ecology* 17 (1): 121–30.

Manzoni, Stefano, Petr Čapek, Maria Mooshammer, Björn D. B.D. Lindahl, Andreas Richter, and Hana Šantrůčková. 2017. "Optimal metabolic regulation along resource stoichiometry gradients." Edited by Dedmer Van de Waal. *Ecology Letters* 20 (9): 1182–91. doi:[10.1111/ele.12815](https://doi.org/10.1111/ele.12815).

Poisot, T. 2011. "The digitize package: extracting numerical data from scatterplots." *The R Journal* 3 (1): 25–26. http://rjournal.github.io/archive/2011-1/RJournal{_}2011-1.pdf{\#}page=25.

Sinsabaugh, Robert L, Stefano Manzoni, Daryl L Moorhead, Andreas Richter, and James Elser. 2013. "Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling." *Ecology Letters* 16 (7): 930–39. doi:[10.1111/ele.12113](https://doi.org/10.1111/ele.12113).

Van Duren, I. C., D. Boeye, and A. P. Grootjans. 1997. "Nutrient limitations in an extant and drained poor fen: implications for restoration." *Plant Ecology* 133 (1). Kluwer Academic Publishers: 91–100. doi:[10.1023/A:1009728007279](https://doi.org/10.1023/A:1009728007279).

Watanabe, Toshihiro, Masaru Urayama, Takuro Shinano, Ryosuke Okada, and Mitsuru Osaki. 2015. "Application of ionomics to plant and soil in fields under long-term fertilizer trials." *SpringerPlus* 4 (1). Springer International Publishing: 781. doi:[10.1186/s40064-015-1562-x](https://doi.org/10.1186/s40064-015-1562-x).

Xu, X F, P E Thornton, and W M Post. 2013. "A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems." *Global Ecology and Biogeography* 22 (6): 737–49. doi:[10.1111/geb.12029](https://doi.org/10.1111/geb.12029).