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**Global response patterns of plant functional traits to combined nitrogen and phosphorus addition are governed by additive interactions**

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**Abstract**

The availability of nutrients such as nitrogen (N) and phosphorus (P) play an important role in shaping plant ecophysiological responses to global change. While nitrogen availability has been asserted as a key driver of plant responses to global change, the role of phosphorus – both individually and in combination with nitrogen – remains less understood. This is due to a lack of broad data syntheses that precludes the development of a mechanistic framework. To address this knowledge gap, we compiled global leaf and whole-plant trait data from full-factorial nitrogen and phosphorus addition experiments across the globe and conducted a meta-analysis. We used this approach to quantify the individual and interactive effects of nitrogen and phosphorus on net photosynthesis, photosynthetic capacity, leaf nutrient content, plant biomass accumulation, and biomass partitioning. Across experiments, nitrogen addition generally increased leaf nitrogen content on both a mass- and area-basis but did not change leaf phosphorus content, leading to an increase in the leaf nitrogen-to-phosphorus ratio. In contrast, phosphorus addition increased leaf phosphorus on a mass- and area-basis but did not change leaf nitrogen content, leading to a decrease in the leaf nitrogen-to-phosphorus ratio. We found no evidence that nitrogen or phosphorus addition influenced net photosynthesis apparent photosynthetic capacity, or photosynthetic nitrogen and phosphorus use effiicencies. Nitrogen and phosphorus addition each increased aboveground biomass and did not alter belowground biomass, leading to a reduction in the root mass fraction and root-to-shoot ratio. An analysis of interaction effect sizes indicated that the combined effects of nitrogen and phosphorus addition on leaf and whole-plant traits were primarily driven by additive interactions, indicating that these responses were generally the result of independent effects of each nutrient addition. These findings show that nitrogen and phosphorus availability additively impact leaf chemistry and biomass but have no effect on leaf-level photosynthesis. In fact, null photosynthetic responses to nutrient additions are supportive of previous work showing that investment in photosynthesis is more strongly regulated by climatic factors that alter demand for soil resources (e.g., CO2, temperature) than by changes in nutrient availability.

**Introduction**

[introduction, eutrophication and global change]

[N addition effects on leaf and whole-plant traits]

[P addition effects on leaf and whole-plant traits]

[Combined N and P effects on leaf and whole-plant traits, including knowledge gaps]

[study objectives]

Here, we conducted a global meta-analysis using [XX] observations from [XX] journal articles, including data compiled from an existing database of plant functional trait responses to nitrogen and phosphorus addition. Our objectives were two-fold. First, we sought to quantify the effects of N, P, and N+P addition on net photosynthesis, photosynthetic capacity, leaf nutrient content and partitioning, resource use efficiencies, plant growth, and biomass partitioning. Second, we quantified the interaction effect size of each trait to understand whether the effects of N+P addition were the product of additive, synergistic, or antagonistic individual effects of N and P addition. We used this approach to test the following hypotheses:

1. Nitrogen and phosphorus addition will increase leaf nitrogen content and leaf phosphorus content, respectively, but will not influence photosynthetic parameters as demand to build and maintain photosynthetic enzymes will not change based on nitrogen or phosphorus availability. Despite this, nitrogen and phosphorus addition will each strongly increase total biomass through stronger increases in aboveground biomass than belowground biomass, which will decrease the root-to-shoot ratio and root mass fraction. We expected that plant functional group would largely regulate the magnitude of species’ responses to nitrogen and phosphorus addition. C4 species, species forming strong associations with microbial symbionts, woody species, and perennial species were each predicted to exhibit weaker responses to nutrient additions than C3 species, species that rely on direct uptake methods, herbaceous species, and annual species
2. The combined effects of nitrogen and phosphorus addition on leaf and whole-plant traits will be the sum of the corresponding individual effects of nitrogen and phosphorus addition. In other words, the combined effects of nitrogen and phosphorus addition will be the product of additive responses, not synergistic or antagonistic.

**Materials and Methods**

*Data compilation*

Initial data for the meta-analysis were collected using citations listed in the Manipulation Experiments Synthesis Initiative (MESI) database (Van Sundert *et al.*, 2023). We selected field manipulation experiments that added N and P in a full-factorial design to ensure that any comparisons made between N, P, and N+P addition responses were from the same subset of experiments. All data for manuscripts included in the MESI database that fit these criteria were downloaded or extracted using a plot digitizer to ensure that all relevant traits were included in the meta-analysis and undergo a round of quality control to avoid any data entry issues that may arise when using large ecological datasets (Augustine *et al.*, 2024). To supplement studies included in the MESI database, studies that reported data from Nutrient Network experiments were also included in the meta-analysis, including only measurements collected from control, N, P, and N+P addition plots. Each site in each paper that reported data from Nutrient Network experiments was treated as an independent experiment, following that the Nutrient Network is a globally distributed experiment where independent sites share the same nutrient addition and experimental design scheme (Borer *et al.*, 2014). Specifically, we added leaf nutrient data from Firn et al. (2019), biomass partitioning data from Cleland et al. (2019), and photosynthetic data from Hersch-Green et al. (2024).

To further supplement MESI and Nutrient Network datasets, we added additional manipulation experiments using journal articles published on or before March 2025. We selected manipulation experiments where N and P were added in a full-factorial design (that is, experiments must have a control, N, P, and N+P treatment). From this, we selected experiments that measured traits related to leaf photosynthesis (e.g., net photosynthesis photosynthetic capacity, stomatal conductance), leaf nutrient content (e.g., mass- or area-based leaf nitrogen content, mass- or area-based leaf phosphorus content), biomass (e.g., above-ground or belowground biomass), biomass partitioning (e.g., root:shoot ratio), or nutrient partitioning of the biomass (e.g., aboveground nitrogen biomass, aboveground phosphorus biomass). Finally, we selected experiments that included explicit explanations of treatment replication schemes to accurately calculate summary statistics. We first searched for studies that followed these guidelines using citations included in the MESI and Nutrient Network papers. To supplement these studies, we also created a search query in Web of Science using similar search terms as in (Liang *et al.*, 2020). Specifically, our query mined for the following topics: (nitrogen AND phosphorus) AND (fertiliz\* OR addition) AND (effect\* OR respon\* OR affect\* OR impact\* OR increas\* OR decreas\* OR alter\* OR deposition OR enrich\*) AND (leaf nitrogen\* OR leaf phosphorus\* OR \*use efficiency OR biomass OR mass fraction OR root:shoot OR LMA OR SLA OR chlorophyll OR photosynthesis OR Vcmax OR Jmax) NOT (animal\* OR medic\* OR chemist\*).

*Data extraction*

For leaf-level measurements, one “mean value ± standard deviation” per trait per species per nutrient fertilization treatment per experiment was considered one “observation”. Observations for different species from the same study were considered independent and allowed us to determine the effects of plant functional group (e.g., mycorrhizal type, photosynthetic pathway, growth form) on plant responses to nutrient fertilization treatments. For whole-plant measurements, one “mean value ± standard deviation” per nutrient fertilization treatment per experiment was considered as one “observation” to account for challenges associated with isolating whole-plant traits to the species-level in the field. Given this, the final dataset used for the meta-analysis included XX observations from XX studies (Table SX).

Data were integrated into a compiled dataset through multiple pathways. First, we manually calculated summary statistics using datasets from studies that adopted open data practices. Next, we sifted studies for summary statistics included in tables included in the main text or supplemental information and included these values directly in our dataset. If studies did not include their data or provide summary statistics in tables, we digitized plots using information about treatment and sample replication information. Plots were digitized in R (version 4.4.2) using the ‘metadigitise’ package (Pick *et al.*, 2019).

*Moderator variables*

All field experiments reported site latitude and longitude coordinates. Using these coordinates, we extracted monthly climate data spanning 1901-2024 using the Climatic Research Unit Time Series gridded data product at a 0.5° resolution (CRU TS v4.09; (Harris *et al.*, 2020). Data were extracted from the grid cell containing each site using the “extract” function in the “raster” R package (Hijmans, 2010). Specifically, we extracted data for monthly average temperature (°C), total monthly precipitation (mm month-1), and total monthly potential evapotranspiration (cm month-1). Mean annual temperature, precipitation, and potential evapotranspiration were calculated for each site by first calculating the mean temperature, total precipitation, and total potential evapotranspiration for each year separately, then calculating the average of these climatic variables across the 1901-2024 period. We used mean annual precipitation and mean annual potential evapotranspiration to calculate the mean annual aridity index (AI, unitless). Low AI values indicate more arid sites. Site climate data are reported in Table XX.

Species identity traits were included for all measurements that were collected at the species level. Specifically, we included information about species family, growth form (tree/shrub, graminoid, forb), growth duration (annual, perennial), photosynthetic pathway (C3, C4), N2-fixation ability (N2-fixer or non-fixer), and mycorrhizal type (AM, EcM, dual AM-EcM, facultative AM, ErM, and non-mycorrhizal). N2-fixation ability was determined based on whether species were in the *Fabaceae* family. Mycorrhizal type was assigned from the FungalRoot database using the genus of each species (Soudzilovskaia *et al.*, 2020).

*Determination and analysis of individual and interaction effect sizes*

We followed an established framework for assessing individual and interactive effects of multiple treatments in meta-analysis (Yue *et al.*, 2017). First, we used the natural logarithm of the response ratio (ln RR) to determine the individual effects of N, P, and N+P addition on leaf and whole-plant traits. For each observation *i* (i.e., trait per species per site per experiment), we calculated the log-response ratio as:

(1)

Where is the mean value of a treatment (i.e., N, P, or N+P addition) and is the mean value of the control treatment. We determined the weighted log-response ratio () of each trait *k* as:

(2)

Where ln RRi is the log-response ratio of observation *i* given in (1), *w*i is the weight of each log-response ratio, and *k* is the total number of observations. *w*i was calculated as the inverse of the variance (*v*i) of observation *i* (that is, *w*i = 1 / *v*i). *v*i was calculated as:

(3)

Where *s*t and *s*c are the standard deviations of the treatment and control groups, respectively, and *n*t and *n*c are the sample sizes of the treatment and control groups.

Next, we used Hedge’s *d* to determine the interactive effect of N and P addition on leaf and whole-plant traits (Yue *et al.*, 2017; Ding *et al.*, 2025). For each observation *i*, the interactive effect size of N and P addition (dNPi) was calculated as:

(4)

Where ,,, and refer to the mean of the N, P, N+P, and control treatments, respectively, for observation *i*. *s*int refers to the pooled standard deviation across treatments, calculated as:

(5)

Where *N*c, *N*n, *N*p, and *N*np refer to the sample sizes of control, N, P, and N+P treatments, respectively. *S*c, *S*n, *S*p, and *S*np refer to the sample sizes of control, N, P, and N+P treatments, respectively. In (6), *J*int refers to a correction term for small sample size bias, calculated as:

(6)

We determined the weighted interaction effect size () of each trait across experiments as:

(7)

Where *d*NPi is the interaction effect size of observation *i* given in (6), *w*dnpi is the associated weight of each interaction effect size, and *k* represents the total number of observations. *w*dnpi was calculated as the inverse of the variance (*vd*NPi) of observation *i* (that is, *w*dnpi = 1 / *vd*NPi). *vd*NPi was calculated as:

(8)

*Data analysis*

We constructed a series of mixed-effects meta-regression models to understand the individual and interactive effects of N and P addition on leaf and whole-plant traits. Three separate models were created for each trait to assess the individual effects of N, P, and N+P addition using log-response ratios and their associated variances. We created a fourth model for each trait to assess the interactive effect of N and P addition using *d*NPi values and their associated variances and weights. In all cases, we built mixed-effects meta-regression models using the ‘rma.mv’ function in the ‘metafor’ R package (Viechtbauer, 2010), manually specifying the weights of each observation as explained above and fitting each model using restricted maximum likelihood estimation. All models included climatic moderator variables (MAT, MAP, AI) and species identity moderator variables (growth form, growth duration, photosynthetic pathway, N2-fixation ability, mycorrhizal status) as fixed effects.

Interactions between N and P addition on leaf and whole-plant traits were classified into three categories: additive, synergistic, and antagonistic. Following

If positive or negative effects of N or P addition corresponded with a null interaction effect (i.e. 95% confidence intervals overlapping with zero), then the combined effect of N and P addition did not have stronger effects than when nutrients were added in isolation, indicating an additive effect. However, if positive individual effects of N or P addition corresponded with a significant positive interaction effect (i.e. the interaction effect size and confidence intervals were all positive), then the combined positive effect of N and P addition was stronger than in isolation, indicating a synergistic interaction. Similarly, if negative individual effects of N or P addition corresponded with a significant negative interaction effect, then the combined negative effect of N and P addition was stronger than when nutrients were added in isolation, also indicating a synergistic effect. Finally, if positive individual effects of N or P addition corresponded with a significant negative interaction effect, then the combined effect of N and P addition was weaker than when nutrients were added in isolation, indicating an antagonistic effect.

**Results**

*Leaf nutrient content*