**Global response patterns of plant functional traits to nitrogen and phosphorus addition are driven by additive individual effects of N and P addition**

**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 well-documented 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 knowledge gap arises in part due to a lack of a mechanistic framework for understanding how P availability influences traits related to photosynthesis, resource use, biomass partitioning, and growth. To address this, 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 used these responses to make inferences about whether the effects of N+P addition are the product of additive, synergistic, or antagonistic individual effects of N and P addition. Meta-analysis results show that P addition increased leaf phosphorus content by XX%, a pattern that was mostly driven by an increase in the XX fractional pool.

**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 used these responses to make inferences about whether the effects of N+P addition are the product of additive, synergistic, or antagonistic individual effects of N and P addition. We used this meta-analytic 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 1) ensure that all relevant traits were included in the meta-analysis and 2) 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 are 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\*).

For leaf-level measurements, one “mean value ± standard deviation” per trait per species per nutrient fertilization treatment per experiment was considered as 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 extraction*

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).

*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 to determine the individual effects of N, P, and N+P addition on leaf and whole-plant traits.

calculated individual effects of N, P, and N+P addition on leaf and whole-plant traits using the natural logarithm of the response ratio, and interaction effects of N and P addition on leaf and whole-plant traits using Hedge’s *d* (Hedges *et al.*, 1999).

We calculated the natural logarithm of the response ratio (ln RR) to assess the individual effects of N, P, and N+P addition on leaf and whole-plant traits. For each observation *i*, ln RR was calculated as:

we calculated individual effects of N, P, and N+P addition on leaf and whole-plant traits using Hedge’s *g* and the interaction effects of N and P addition using Hedge’s *d* (Yue *et al.*, 2017; Ding *et al.*, 2025).

~~To determine the interaction effect of N and P addition on leaf and whole-plant traits, we used Hedge’s~~ *~~g~~*~~. For each observation~~ *~~i~~*~~, the individual effect size (~~*~~g~~*~~i~~~~) was calculated as:~~

~~(1)~~

~~refers to the mean value of a treatment (N, P, or N+P) and refers to the mean of the control treatment.~~ *~~s~~*~~t~~ ~~refers to the pooled standard deviation, calculated as:~~

~~(2)~~

~~where~~ *~~N~~*~~c~~ ~~is the sample size of the control treatment,~~ *~~N~~*~~t~~ ~~is the sample size of a treatment (N, P, or N+P),~~ *~~S~~*~~c~~ ~~is the standard deviation of the control treatment, and~~ *~~S~~*~~t~~ ~~is the standard deviation of the treatment (N, P, or N+P). In Equation 1,~~ *~~j~~*~~t~~ ~~is a correction term for small sample size bias, where:~~

~~(3)~~

~~We determined the weighted effect size (~~*~~g~~*~~) of each trait across experiments as:~~

~~(4)~~

~~Where~~ *~~g~~*~~i~~ ~~is the effect size of observation~~ *~~i~~* ~~given in (1),~~ *~~w~~*~~i~~ ~~is the associated weight of each effect size, and~~ *~~k~~* ~~represents the total number of observations.~~ *~~w~~*~~i~~ ~~was calculated as the inverse of the variance (~~*~~vg~~~~i~~*~~) of observation~~ *~~i~~* ~~(that is,~~ *~~w~~*~~i~~ ~~= 1 /~~ *~~vg~~*~~i~~~~).~~ *~~vg~~~~i~~* ~~was calculated as:~~

(5)

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:

(6)

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:

(7)

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:

(8)

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

(9)

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:

(10)

Finally, 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 moeta-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.

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