**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 papers included in the MESI database, data published from studies part of the Nutrient Network were included in the meta-analysis, including only measurements collected from control, N, P, and N+P addition plots. Each site in the Nutrient Network database was treated as an independent experiment, following that the Nutrient Network is a globally distributed experiment where independent sites share a similar 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). We then added a series of additional field manipulation experiments using journal articles published on or before March 2025. To compile these experiments, we created a complex query in Web of Science, using similar search terms as used 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 Vcmax OR Jmax) NOT (animal\* OR medic\* OR chemist\*). Search results for this query yielded 9,234 articles, which were distilled to satisfy the following criteria:

1. Field manipulation experiment where N and P are added in a full-factorial design (that is, experiments must have a control, N, P, and N+P treatment). The dosage of N and P in isolation must be the same dosage of N and P included in the N+P treatment.
2. Experiment must measure 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 (e.g., aboveground nitrogen biomass, aboveground phosphorus biomass).
3. Experiment must report the treatment replication scheme to extract experiment summary statistics. This can be included directly in the paper or implicitly if data were publicly available.
4. Experiment must report the duration of the fertilization treatments.

These criteria rendered the addition of XX additional studies. The final dataset included measurements from XX studies (Table SX).

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. Due to the difficulty associated with isolating whole-plant traits (e.g., belowground biomass) in the field to the species-level (e.g., below-ground biomass), one “mean value ± standard deviation” per nutrient fertilization treatment per experiment was considered as one “observation”.

*Data extraction*

For observations included in the MESI database, means, standard deviations, and sample sizes of the control and nutrient addition treatment groups were readily available and used directly in the meta-analysis. For observations in the Nutrient Network data compilation, summary statistics for the control and nutrient addition treatment groups were determined for each trait for each species at each site. Summary statistics for Firn et al. (2019) and Hersch-Green et al. (2024) were determined for each treatment such that there was one mean, standard deviation, and sample size value for each each treatment for each trait for each species for each site.

the Nutrient Network data were pooled across species, such that there was one mean, standard deviation, and sample size for each treatment within each trait for each site. For additional experiments added through Web of Science, we calculated summary statistics for the control and nutrient addition treatment groups for each trait, again pooling the statistics across species such that there was one mean, standard deviation, and sample size value for each treatment.

Due to an abundance of articles that did not publish data alongside the article, we used the ‘metaDigitise’ R package to estimate summary statistics from plots when articles included sample sizes (Pick et al., 2019). A column is included in our compiled dataset that notes whether summary statistics were determined using actual data or estimates from plots.

*Data analysis*

The individual effect of nutrient treatments on leaf and whole-plant traits were calculated using the natural logarithm of the response ratio, calculated as:

Where *X*t is the experimental treatment mean and *X*c is the control mean.