**Title**: Increasing nitrogen availability increases water use efficiency and decreases nitrogen use efficiency in *Acer saccharum*: a test of photosynthetic least-cost theory in mature forests

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**Status:** Currently in review at *Journal of Ecology*

**Abstract**

Photosynthesis links terrestrial carbon, water, and nutrient cycles. Photosynthetic least-cost theory suggests that plants optimize photosynthesis by maximizing the use of available light at the lowest summed nutrient and water use. The theory predicts that increasing nutrient availability will enable plants to increase nutrient investment in photosynthetic enzymes, allowing similar photosynthetic rates to be achieved at a lower ratio of leaf intercellular to atmospheric CO2 concentrations (*χ*). The theory suggests similar responses to increasing soil pH in acidic conditions. However, empirical tests of the theory outside of environmental gradients are rare, limiting inferences about mechanisms driving such responses. We measured photosynthetic traits in mature *Acer saccharum* (sugar maple) trees growing in a nine-year, nitrogen-by-pH manipulation experiment in the northeastern United States to test this theory. There was no effect of nitrogen availability on net photosynthesis; however, increasing nitrogen availability was associated with an increase in area-based leaf nitrogen content, a weak increase in photosynthetic capacity, and a weak decrease in *χ*. These patterns resulted in strong positive effects of increasing nitrogen availability on area-based leaf nitrogen content per unit *χ* and increased photosynthetic capacity per unit *χ*. When examined across all plots, soil pH had no effect on any physiological traits. However, in plots without nitrogen additions, increasing soil pH increased area-based leaf nitrogen content and photosynthetic capacity per unit *χ*, though did not directly modify *χ*. These results support nutrient-water use tradeoffs expected from theory, indicating that *A. saccharum* maintained net photosynthesis rates across the nitrogen availability gradient by trading less efficient nitrogen use for more efficient water use. Results also indicate that effects of soil pH on nitrogen-water use tradeoffs occur through pH effects on nitrogen availability. This work can be used to better understand and predict mechanisms driving leaf nitrogen-photosynthesis relationships across resource availability gradients.

**Title**: The cost of resource use for photosynthesis drives variance in leaf nitrogen content in Texan grasslands

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**Abstract**

Climate and resource availability are important drivers of plant nitrogen uptake and allocation. Photosynthetic least cost theory provides a unified framework for understanding the integrative role of climate and soil resource availability on leaf nitrogen content, positing that water and nitrogen can be used as substitutable resources to support photosynthesis. The theory also indicates that leaf investment in water use (reflected in the ratio of leaf intercellular to atmospheric CO, *C*i:*C*a) or nitrogen use (reflected in area-based leaf nitrogen content, *N*area) are each a function of the unit cost of acquiring and using nitrogen relative to water (*β*) and aboveground climate, which alters demand for water and nitrogen. While promising, no study to date has explicitly tested this theory by concurrently measuring *β*, leaf *C*i:*C*a, and *N*area (and its components: leaf mass per area, *M*area; leaf nitrogen per unit leaf biomass, *N*mass) across environmental gradients. To test the theory, *N*area, *M*area, *N*mass, leaf *C*i:*C*a, and *β* were measured in 499 individuals comprising 52 species across 23 sites scattered along a climatic and resource availability gradient in Texas, USA. Across the gradient, *N*area increased with increasing nitrogen availability and soil moisture, but decreased with increasing leaf *C*i:*C*a. The negative relationship between leaf *C*i:*C*a and *N*area was driven by negative covariance between leaf *C*i:*C*a and *M*area coupled with no relationship between leaf *C*i:*C*a and *N*mass, suggesting that nitrogen-water use tradeoffs were modified through changes in leaf morphology and were not the product of changes in leaf chemistry per se. Increasing VPD indirectly increased *N*area when mediated through a negative effect of increasing VPD on leaf *C*i:*C*a and negative relationship between leaf *C*i:*C*a and *M*area. These results support patterns expected from photosynthetic least-cost theory, demonstrating the capability of the theory to predict the integrative role of edaphic and climatic factors on leaf nitrogen content.

**Title**: Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO2 at different scales

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**Abstract**

Plants respond to elevated atmospheric CO2 concentrations by reducing leaf nitrogen content, stomatal conductance, and photosynthetic capacity. These responses coincide with increased growth rates and total leaf area over short time scales that dampen with time. Nitrogen supply has been hypothesized to be the primary factor controlling these responses, as nitrogen availability limits net primary productivity globally. Recent work calls aspects of this hypothesis into question, suggesting that leaf responses to elevated CO2 are independent of nitrogen supply and are instead determined by demand to build and maintain photosynthetic machinery, which optimizes resource allocation to photosynthetic capacity. To reconcile the role of nitrogen supply and demand on leaf and whole-plant responses to elevated CO2, we grew *Glycine max* L. (Merr) seedlings under one of two CO2 concentrations, with and without inoculation with *Bradyrhizobium japonicum*, and across nine nitrogen fertilization treatments in a full-factorial growth chamber experiment. After seven weeks, elevated CO2 reduced photosynthetic capacity independent of fertilization or inoculation treatment. Despite this, positive effects of elevated CO2 on total biomass and total leaf area were enhanced with increasing fertilization, patterns that were associated with increased whole-plant nitrogen uptake. Positive effects of elevated CO2 on whole-plant growth were enhanced in inoculated plants, though this pattern was only apparent under low fertilization. Results indicate that nitrogen supply and demand each control plant responses to elevated CO2 but operate on different scales. Additionally, patterns indicate that optimal resource allocation to photosynthetic capacity under elevated CO2 may result in nitrogen savings at the leaf level that could alleviate nitrogen limitation at the whole-plant level. The differential role of fertilization on leaf and whole-plant responses to elevated CO2 build on previous work suggesting that terrestrial biosphere models may improve the simulation of photosynthetic processes under future, novel environments by adopting frameworks that include optimality principles.