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Dear Editorial Board at *Journal of Ecology*,

Please find our manuscript titled “The cost of resource use for photosynthesis drives variation in leaf nitrogen content across a climate and resource availability gradient” for consideration as a Full Research Article at the *Journal of Ecology*. The manuscript contains six tables and five figures, accompanied by two tables and two figures as supplemental material.

This study explores the influence of climate and resource availability on leaf nitrogen content across a resource availability and precipitation gradient in grasslands of Texas, USA. We investigate patterns expected from photosynthetic least-cost theory, which offers insights into understanding how climatic and soil resource availability affect leaf nitrogen content and photosynthetic capacity. This theory posits that leaf investment in water (indexed by the ratio of intercellular CO2 to atmospheric CO2, or leaf *C*i:*C*a) and nutrient use (indexed by area-based leaf nitrogen content) are functions of the unit cost of acquiring nitrogen relative to water (*β*) and climate, which alters demand for water and nitrogen to support photosynthesis. Despite efforts to incorporate this theory into land surface models, empirical support of the theory remains limited, and no study to date has assessed patterns expected from the theory across environmental gradients using concurrently measurements of *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). To bridge this knowledge gap, we measured *N*area, *N*mass, *M*area, leaf *C*i:*C*a, and *β* in 515 individuals comprising 57 species with an environmental gradient experiment conducted in grasslands of Texas, USA.

Patterns consistent with those expected from photosynthetic least-cost theory emerged, a result driven by a negative relationship between leaf *C*i:*C*a and *N*area mediated through a direct negative effect of increasing leaf *C*i:*C*a on *M*area coupled with no relationship between leaf *C*i:*C*a on *N*mass. In further support of the theory, increasing nitrogen availability decreased *β*, resulting in two pathways that contributed to indirect positive effects of increasing nitrogen availability on *N*area: (1) when mediated through a negative effect of increasing nitrogen availability on *β*, a positive relationship between *β* and leaf *C*i:*C*a, and negative effect of increasing leaf *C*i:*C*a on *M*area, and (2) when mediated through a negative effect of increasing nitrogen availability on *β* and negative effect of increasing *β* on *N*mass. Results indicated a third pathway where increasing nitrogen availability increased *N*area directly through a larger increase in *N*mass than decrease in *M*area independent of *β* or leaf *C*i:*C*a, suggesting that the theory was capable of predicting some, but not all, variance in leaf nitrogen content due to nitrogen availability. Increasing vapor pressure deficit indirectly increased *N*area when mediated through a negative effect of increasing vapor pressure deficit on leaf *C*i:*C*a and negative relationship between leaf *C*i:*C*a and *M*area.

Findings from this study provide important insight into understanding drivers of variability in leaf nitrogen content across environmental gradients. Our study addresses a timely and significant knowledge gap in plant functional ecology, as previous research investigating patterns expected from photosynthetic least-cost theory has been focused on understanding variance in *N*area across environmental gradients, but has largely ignored components of *N*area, *N*mass and *M*area. Here, we show that quantifying variability in *N*mass and *M*area can provide useful insight toward understanding the mechanisms governing *N*area across environmental gradients. Additionally, past studies have focused on understanding patterns expected from the theory at the global scale. Our findings suggest that such patterns also occur at finer spatial scales, demonstrating the wide applicability of this theory across ecological scales.

We believe this manuscript will be received well by the plant ecophysiological and modeling communities due to its novel empirical approach and use of a theory actively being implemented in next-generation terrestrial biosphere models. This article is a good fit for the broad readership at the *Journal of Ecology* due to its interdisciplinary approach and because the *Journal of Ecology* has previously published manuscripts investigating patterns expected from photosynthetic least-cost theory.

Please do not hesitate to contact me at the e-mail address listed above over any comments or concerns.

Sincerely,

Evan A. Perkowski, Ph.D.

*On behalf of coauthors Helen G. Scott and Nicholas G. Smith*