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Dear Editorial Board at *Journal of Ecology*,

Please find our manuscript titled “The cost of resource use for photosynthesis drives variance in leaf nitrogen content in grasslands of Texas, USA” 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.

Climate and resource availability are important predictors of variance in leaf nitrogen content across environmental gradients.

Our study explores the influence of climate and resource availability on leaf nitrogen content across environmental gradients. We investigate patterns expected from photosynthetic least-cost theory, which offers insights into how climatic and soil factors affect leaf nitrogen content and photosynthetic capacity. This theory posits that plants acclimate to their environment by minimizing the summed cost of acquiring nitrogen relative to water. Despite recent efforts to incorporate this theory into land surface models, empirical support remains limited across environmental gradients. To address this gap, we measured area-based leaf nitrogen content (Narea), its components (mass-based leaf nitrogen content, Nmass; leaf mass per area, Marea), the leaf intercellular CO2 to atmospheric CO2 ratio (leaf Ci:Ca), and the unit cost to acquire nitrogen relative to water (β) in 499 individuals comprising 52 species in grasslands of Texas, USA.

Patterns supporting 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. In further support of the theory, increasing nitrogen availability was negatively associated with *β*, resulting in two pathways that contributed to an indirect positive effect 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 Narea directly through a larger increase in Nmass than decrease in Marea independent of changes in β or leaf Ci:Ca. Findings from this study provide important insight into understanding drivers of variability in leaf nitrogen content across environmental gradients and suggest that optimality models may improve model simulations by including an approach for predicting β dynamically across environmental gradients.

Our study addresses a timely and significant gap in plant functional ecology, as previous research has mainly focused on Narea and has largely ignored its constituent parts, Nmass and Marea. Here, we show that understanding variability in *N*mass and *M*area across environmental gradients can provide useful insight toward understanding the mechanisms governing *N*area. Additionally, past studies supporting theoretical expectations have largely been conducted at global scales. Our findings suggest that such patterns occur at finer scales, demonstrating the wide applicability of this theory across ecological scales.

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*