Evan A. Perkowski, Ph.D.

Postdoctoral Research Associate

Dept. of Biological Sciences

Texas Tech University

2901 Main St.

Lubbock, TX 79409

[Evan.a.perkowski@ttu.edu](mailto:Evan.a.perkowski@ttu.edu)

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Dear Editorial Board at *New Phytologist*,

Please consider the attached manuscript, titled “Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO2 at different scales” for consideration as a full research article to *New Phytologist*. The manuscript contains 3 tables and 3 figures embedded in the main text with 6 tables and 6 figures included as supplemental material.

1. **What hypotheses or questions does this work address?**

The manuscript describes results from a growth chamber experiment in which Glycine max L. (Merr) seedlings were grown under full-factorial combinations of two CO2, two inoculation, and nine nitrogen fertilization treatments. After seven weeks of vegetative growth, we assessed gas exchange and growth responses to treatment combinations. A central goal of the experiment was to reconcile the role of nitrogen availability and nitrogen demand on leaf- and whole-plant responses to elevated CO2 using patterns expected from the progressive nitrogen limitation hypothesis and eco-evolutionary optimality theory. Specifically, the manuscript tests the hypothesis that nitrogen availability and demand will each determine plant responses to elevated CO2, predicting that each will operate at different levels of organization. Following eco-evolutionary optimality theory, we expected that nitrogen demand for building and maintaining photosynthetic enzymes would drive leaf photosynthetic responses to elevated CO2 independent of nitrogen availability. In contrast, we expected that nitrogen availability would regulate whole-plant responses to elevated CO2 due to increased plant nitrogen uptake and reduced nitrogen acquisition costs, following patterns expected by progressive nitrogen limitation.

1. **How does this work advance our current understanding of plant science?**

Our results reconcile the role of nitrogen availability and demand on plant responses to elevated CO2, showing that nitrogen demand for building and maintaining photosynthetic enzymes dictate leaf photosynthetic responses to elevated CO2 and nitrogen availability regulate whole-plant responses.

Supporting our hypotheses, nitrogen fertilization did not modify leaf photosynthetic responses to elevated CO2. Instead, elevated CO2 decreased the maximum rate of Rubisco carboxylation more strongly than it decreased the maximum rate of electron transport for RuBP regeneration, increasing net photosynthesis rates by approaching optimal coordination and maximizing nutrient use efficiency. In further support of our hypotheses, positive growth responses to elevated CO2 were enhanced with increasing fertilization

1. **Why is this work important and timely?**

Photosynthesis represents the largest carbon flux between the atmosphere and biosphere and is regulated by complex carbon and nitrogen cycles. Terrestrial biosphere models are sensitive to the formulation of photosynthetic processes, yet often model photosynthetic responses to increasing atmospheric CO2 concentrations as a function of progressive nitrogen limitation. Our results contradict this framework, indicating that photosynthetic responses to elevated CO2 are independent of nitrogen availability and are driven by patterns expected by eco-evolutionary optimality theory. This work is therefore timely and important as it suggests that terrestrial biosphere models may improve the accuracy by which photosynthetic processes are modeled under novel growth environments by implementing optimality principles.

Please do not hesitate to contact me with the contact information below over any questions and concerns. Please note that a version of this manuscript is currently available as a pre-print to *bioRxiv* (DOI: <https://doi.org/10.1101/2023.11.30.567584>)

Sincerely,

Evan Perkowski

*On behalf of Ezinwanne Ezekannagha and Nick Smith*