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)

January 18, 2024

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 three tables and three figures embedded in the main text, with six tables and six 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 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. We also expected that investment in symbiotic nitrogen fixation would increase under elevated CO2, increasing whole-plant responses to elevated CO2. However, we expected this response to only be apparent under low nitrogen fertilization, where individuals would invest more strongly in the symbiosis.

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 dictates leaf photosynthetic responses to elevated CO2 and that nitrogen availability regulates whole-plant responses. Specifically, we found that nitrogen fertilization and inoculation 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. Nitrogen fertilization enhanced positive whole-plant growth responses to elevated CO2 due to enhanced nitrogen uptake and reduced costs of nitrogen acquisition. Interestingly, inoculation did not affect plant responses to elevated CO2 due to similar plant investments toward nitrogen fixation between CO2 treatments. These patterns largely supported our hypotheses, showing that nitrogen availability and demand for building and maintaining photosynthetic enzymes determine plant responses to elevated CO2, operating at different organizational levels. To our knowledge, this is the first study to use concurrent leaf and whole-plant measurements to explicitly reconcile diverging hypotheses regarding the role of nitrogen availability on plant responses to elevated CO2, providing critical insight for modeling these processes with greater accuracy and reliability under future novel environments.

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

Photosynthesis is 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 often model photosynthetic responses to increasing atmospheric CO2 concentrations as a function of progressive nitrogen limitation. Our results contradict this framework, indicating that patterns expected by eco-evolutionary optimality theory drive leaf photosynthetic responses to elevated CO2 independent of nitrogen availability.

This work is timely and important as it suggests that terrestrial biosphere models may improve the accuracy by which photosynthetic processes are modeled under future novel growth environments by implementing optimality principles.

Please contact me using the information below for any questions or concerns. This manuscript is not currently submitted or in review at any other journal. Please note that a version of this manuscript is currently available as a pre-print on *bioRxiv* (DOI: <https://doi.org/10.1101/2023.11.30.567584>).

Sincerely,

Evan A. Perkowski, Ph.D.

*On behalf of Ezinwanne Ezekannagha and Nicholas G. Smith*