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Dear Editorial Board at *Plant, Cell & Environment*,

Please consider the attached manuscript, titled “Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO2”, for consideration as a full research article to *Plant, Cell & Environment*. The manuscript contains three tables and three figures embedded in the main text, with seven tables and seven figures included as supplemental material.

Terrestrial biosphere model simulations of the future land carbon sink are sensitive to the representation of photosynthetic processes and their response to increasing CO2 concentrations. Current approaches for simulating photosynthetic responses to CO2 in terrestrial biosphere models often invoke patterns expected from nitrogen limitation, where nitrogen availability diminishes with time due to increasing CO2 concentrations because whole-plant nitrogen demand exceeds supply, depleting the pool of available nitrogen for plants to acquire and allocate to new tissues. This response causes models to simulate a reduction in leaf nitrogen content and therefore photosynthetic capacity, as leaf-level photosynthesis is commonly modeled as a function of positive relationships between nitrogen availability, leaf nitrogen content, and photosynthetic capacity. However, recent work using eco-evolutionary optimality theory suggests that photosynthetic responses to elevated CO2 decrease demand to build and maintain photosynthetic enzymes, which results in an allocation response that optimizes resource allocation to photosynthetic capacity and maximizes allocation to growth independent of soil nitrogen availability.

Here, for the first time, we provide experimental evidence that reconciles these competing hypotheses, forging a path forward for implementing improved representations of plant responses to elevated CO2 across soil nitrogen availability gradients in terrestrial biosphere models. Using a growth chamber experiment that grew *Glycine max* L. (Merr) seedlings under full-factorial combinations of two CO2, two inoculation, and nine nitrogen fertilization treatments, we show 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, our results indicate that nitrogen fertilization and inoculation did not modify reductions in photosynthetic capacity in response 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 allowing rate-limiting steps to approach optimal coordination. Nitrogen fertilization enhanced positive whole-plant growth responses to elevated CO2 due to enhanced nitrogen uptake that reduced costs of nitrogen acquisition. Interestingly, inoculation did not affect leaf or whole-plant responses to elevated CO2.

We feel that results reported from this study would be of great interest to the broad readership at *Plant, Cell & Environment*, as primary conclusions provide a novel contribution to the plant ecophysiological and modeling community that will help improve our ability to simulate and predict land surface carbon-nitrogen interactions under future novel environments. We recommend Kristine Crous ([k.crous@westernsydney.edu.au](mailto:k.crous@westernsydney.edu.au)), Amelia Wolf ([amywolf@utexas.edu](mailto:amywolf@utexas.edu)), and Lucas Cernusak or David Tissue (both members of the *PC&E* Editorial Review Board) as reviewers for this manuscript.

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>), and data are publicly available in a data repository on Zenodo (DOI: [10.5281/zenodo.10162268](https://zenodo.org/doi/10.5281/zenodo.10162268)).

Please contact me using the information above for any questions or concerns.

Sincerely,

Evan A. Perkowski, Ph.D.

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