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January 08, 2024

Dear Editorial Board at *Nature Communications*,

I am writing to submit our manuscript, titled "Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO_2 at different scales", for consideration as an article in *Nature Communications*.

Land surface model simulations of the future land carbon sink are particularly sensitive to the representation of photosynthetic processes and their response to increasing CO_2 concentrations. Models that include photosynthetic acclimation to elevated CO_2 simulate a downregulation in leaf nitrogen content and photosynthetic capacity that results from progressive soil nitrogen limitation, an effect that reduces the future terrestrial carbon sink compared to models that do not simulate this acclimation response. However, recent work using eco-evolutionary optimality theory suggests that photosynthetic responses to elevated CO_2 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 CO_2 across soil nitrogen availability gradients in land surface models.

The manuscript reports findings from a growth chamber experiment where *Glycine max* L. (Merr) seedlings were grown under two CO_2 concentrations (420 ppm and 1000 ppm), nine soil nitrogen fertilization treatments, two inoculation treatments (with and without inoculation with *Bradyrhizobium japonicum*) in a full-factorial design. Net photosynthesis rates, maximum rates of Rubisco carboxylation and electron transport for RuBP regeneration, total leaf area, whole-plant biomass, structural carbon costs to acquire nitrogen, and plant investment toward symbiotic nitrogen fixation were measured after seven weeks of vegetative growth.

Our results indicate that elevated CO_2 decreased the maximum rate of Rubisco carboxylation more strongly than it decreased the maximum rate of electron transport for RuBP regeneration, allowing increased net photosynthesis rates to be achieved through increasingly optimal coordination of Rubisco carboxylation and electron transport for RuBP regeneration. Leaf photosynthetic responses to CO_2 were independent of fertilization or inoculation treatment. Increasing nitrogen fertilization enhanced positive effects of elevated CO_2 on total leaf area and total biomass, responses that were associated with enhanced nitrogen uptake efficiency and reduced carbon costs to acquire nitrogen. Inoculation did not affect whole-plant responses to elevated CO_2 due to similar plant investment toward symbiotic nitrogen fixation between CO_2 treatments. Findings from this study support both the progressive nitrogen limitation and eco-evolutionary optimality hypotheses, suggesting that each hypothesis operates at a different scale. Specifically, leaf photosynthetic responses to elevated CO_2 were strongly indicative of patterns expected from eco-evolutionary optimality theory, while whole-plant responses were constrained by changes in soil nitrogen availability.

Results reported in this manuscript would be of great interest to the broad readership of *Nature Communications*, as primary conclusions provide a novel and potentially paradigm-shifting contribution to ecophysiological and modeling communities that will improve our ability to simulate and predict land surface carbon-nitrogen interactions under future novel environments. This article was submitted as a pre-submission inquiry to *Nature*, who recommended *Nature Communications* as a suitable journal choice.

This manuscript is not currently submitted or in review at any other journal, though a version of the manuscript is uploaded on the *bioRxiv* pre-print server (DOI: 10.1101/2023.11.30.567584). Finally, all data and analyses included in this manuscript are publicly available on Zenodo (DOI: 10.5281/zenodo.10162268).

Please do not hesitate to contact me at the e-mail listed above with any questions or concerns.

Sincerely,

Evan A. Perkowski, Ph.D.

On behalf of coauthors Ezinwanne Ezekannagha and Nicholas G. Smith