Evan A. Perkowski

Department of Biological Sciences

Texas Tech University

Lubbock, TX USA

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

March XX, 2023

Dear Editorial Board at *Nature*,

Atmosphere-biosphere carbon fluxes diverge across terrestrial biosphere models when simulated using future climatic scenarios. Studies from the past decade have shown that models are particularly sensitive to the simulation of photosynthetic processes in response to increasing temperature and CO2 concentration, yet few models incorporate frameworks for simulating plant acclimation responses to such environmental changes.

Over thirty years of experimentation in the plant ecophysiological community has led to a consensus in the community that leaf acclimation responses to increasing CO2 are earmarked by reductions in stomatal conductance and photosynthetic capacity despite apparent increases in net photosynthesis rates. These leaf responses correspond with strong increases in whole plant growth. Some studies show that the strong increase in whole plant growth dampens with time, speculating that this response may be driven by progressive declines in soil nitrogen availability associated with increased demand to acquire and use nitrogen to support net primary productivity. Such results imply that progressive declines in soil nitrogen availability are the mechanism that drives reductions in stomatal conductance and photosynthetic capacity, as an individual leaf might respond more rapidly to changes in soil nitrogen availability than an entire plant. However, this response does not explain why newly expanded leaves often have increased net photosynthesis rates despite reduced photosynthetic capacity and stomatal conductance.

An alternative hypothesis based off optimal coordination theory posits that the downregulation in photosynthetic capacity and stomatal conductance is an allocation strategy that allows plants to maximize resource use efficiency at the leaf level and maximize nutrient allocation to structures that support whole plant growth. The theory predicts that leaves reduce nutrient allocation to Rubisco carboxylation, which allows net photosynthesis to become optimized such that it is equally co-limited by the maximum rate of Rubisco carboxylation and RuBP regeneration.

Progressive nitrogen limitation has received some support in field and pot experiments, but is not consistently observed and is namely driven by increased rates of plant nitrogen uptake. Patterns expected from optimal coordination theory have also received

To disentangle effects of soil nitrogen availability and nitrogen acquisition strategy on leaf and whole plant responses to elevated CO2, we grew *Glycine max* (“soybean”) under one of two CO2 concentrations (420 ppm and 1000 ppm), one of two inoculation treatments (inoculated and uninoculated), and one of nine soil nitrogen fertilization treatments in a full-factorial growth chamber experiment. We harvested all experimental plants after seven weeks of development and measured several leaf whole plant traits. Specifically, we measured leaf nitrogen content, conducted net photosynthesis-by-intercellular CO2 concentration curves to estimate the maximum rates of Rubisco carboxylation and RuBP regeneration, total leaf area, and whole plant biomass. We also calculated the fraction of leaf nitrogen allocated to photosynthesis, structural carbon costs to acquire nitrogen, and the percent of leaf nitrogen acquired from the atmosphere.

In support of patterns expected from optimal coordination theory, we find that elevated CO2 decreased the fraction of leaf nitrogen content allocated to photosynthetic tissue, the maximum rate of Rubisco carboxylation, and the maximum rate of RuBP regeneration. We also find that elevated CO2 decreases the maximum rate of Rubisco carboxylation more than the maximum rate of RuBP regeneration, allowing leaves to approach optimal co-limitation of Rubisco carboxylation and RuBP regeneration rates. In all cases, leaf responses to CO2 were independent of changes in fertilization or inoculation, suggesting that leaf acclimation responses were independent from nitrogen availability. Interestingly, we also find that elevated CO2 increased total leaf area and total biomass, responses that were enhanced with increasing soil nitrogen fertilization and associated with reductions in the cost of acquiring nitrogen and consequent increases in nitrogen uptake.

Interestingly, our results provide support for both the progressive nitrogen limitation and optimal coordination hypotheses, but suggest that each hypothesis operates on a different scale. Specifically, the increase in whole plant growth under elevated CO2 was likely driven by an increase in plant nitrogen uptake, showing support for patterns one might expected from progressive nitrogen limitation. However, soil nitrogen availability did not modify leaf responses to elevated CO2, and leaf physiological responses show strong evidence in support of optimal coordination theory. While this is a pot experiment with apparent limitations in extrapolating patterns to natural ecological systems, findings from this experiment provide a useful mechanism for understanding plant responses to CO2 and provide a framework for better understanding these responses in the field.

Due to the novelty and timeliness of this research, we feel this paper will reach a broad audience and will be received well by both modeling and ecophysiological communities and will be well cited amongst peers. As such, I submit this letter as pre-submission inquiry to *Nature* and the associated *Nature* family of journals to gauge interest in findings from this experiment. Please to not hesitate to contact me with any further questions regarding this study.

Sincerely,

Evan A. Perkowski

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