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Dear Editorial Board at *Nature*,

Land surface model simulations of the future land carbon sink are particularly sensitive to the representation of photosynthetic processes and their response and acclimation to increasing CO2 concentration1. Models that include photosynthetic acclimation to elevated CO2 simulate a downregulation in leaf nitrogen content and photosynthetic capacity that results from progressive soil nitrogen limitation2,3, an effect that reduces the future terrestrial carbon sink compared to models that do not simulate this downregulation4,5. However, others have proposed that this observed downregulation is the result of optimal resource use that maximizes photosynthetic efficiency and plant growth6,7. Here, for the first time, we provide experimental results to reconcile these competing hypotheses, forging a path forward for implementing improved representations of plant responses to elevated CO2 under varying soil nitrogen in land surface models.

The progressive nitrogen limitation hypothesis predicts that elevated CO2 increases plant nitrogen demand to build and maintain photosynthetic tissue, which increases plant nitrogen uptake rates and causes soil nitrogen availability to progressively decline over time3. The hypothesis predicts that declines in soil nitrogen availability will decrease leaf nitrogen and photosynthetic capacity and that increases in total leaf area and biomass will dampen as nitrogen availability becomes more limited. However, progressive nitrogen limitation does not explain why newly expanded leaves often have increased operational net photosynthesis rates despite reduced photosynthetic capacity, and limited empirical evidence exists supporting such an integrated role of soil nitrogen availability on concurrent leaf and whole plant responses to elevated CO28.

An alternative hypothesis to explain plant responses to elevated CO2 suggests that the reduction in leaf nitrogen and photosynthetic capacity is the result of an allocation strategy where C3 plants optimize resource use efficiency at the leaf level, maximizing relative nitrogen allocation to structures supporting whole plant growth. This is because, in C3 plants, fewer photosynthetic enzymes are needed to maximize light use for photosynthesis as a result of reduced photorespiration under elevated CO29,10. If true, the theory predicts that C3 plants should optimally respond to elevated CO2 by decreasing leaf nitrogen content while maintaining enhanced rates of net photosynthesis. This allocation strategy at the leaf level allows plants to maximize nitrogen allocation to whole plant growth. The expected optimal leaf response to elevated CO2 has recently received some empirical support6,7, though no studies have connected these patterns with concurrently measured whole-plant responses to elevated CO2. Importantly, this theory suggests that leaf responses to elevated CO2 are independent of soil nitrogen availability, though does not discount potential positive role of soil N availability on whole plant growth responses to elevated CO2.

To disentangle effects of soil N availability on leaf and whole plant responses to elevated CO2, we grew *Glycine max* under one of two CO2 concentrations (420 ppm and 1000 ppm), one of nine soil N fertilization treatments, one of two inoculation treatments (inoculated and uninoculated with *Bradyrhizobium japonicum* in sterile soil) in a full-factorial growth chamber experiment. After seven weeks of vegetative growth, we measured leaf nitrogen content, 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 photosynthetic and structural tissue11, structural carbon costs to acquire nitrogen12, and investment to symbiotic nitrogen fixation13.

We find that elevated CO2 decreased leaf nitrogen content, the maximum rate of Rubisco carboxylation, and the maximum rate of RuBP regeneration. Elevated CO2 decreased the maximum rate of Rubisco carboxylation more strongly than the maximum rate of RuBP regeneration, allowing leaves to approach optimal co-limitation of Rubisco carboxylation and RuBP regeneration rates by optimizing nutrient allocation to photosynthetic enzymes. In all cases, leaf responses to CO2 were independent of fertilization or inoculation treatment, negating patterns expected from progressive nitrogen limitation. Interestingly, elevated CO2 increased total leaf area and total biomass, responses that were enhanced with increasing fertilization and were associated with reductions in the cost of acquiring nitrogen and increases in nitrogen uptake. We also found that the positive effect of elevated CO2 on total leaf area and total biomass was generally stronger in inoculated plants, though this effect was only apparent under low fertilization due to heavy investment in root nodulation and symbiotic nitrogen fixation that diminished with increasing fertilization.

Our results provide support for both the N limitation and optimal coordination hypotheses, though suggest that each hypothesis operates at a different scale. Specifically, leaf acclimation responses to elevated CO2 were indicative of patterns expected from optimal resource allocation to photosynthetic capacity, while whole plant responses to elevated CO2 were indicative of patterns expected from progressive nitrogen limitation. Importantly, our results suggest that optimal coordination likely results in nitrogen savings at the leaf level that can alleviate progressive N limitation at the whole-plant level.

Elevated CO2 experiments rarely quantify both leaf and whole plant responses concurrently within the same experiment, and studies linking these responses often rely on meta-analyses from different studies14,15. Our findings integrate both leaf and whole-plant responses to elevated CO2 and relate these findings to plant nitrogen uptake rates and costs of nitrogen acquisition. Therefore, we feel this paper provides a novel, potentially paradigm-shifting contribution that substantially improves our understanding and ability to predict future land surface carbon-nitrogen interactions. Given this, we feel that this manuscript would be of great interest to the broad readership of *Nature*. Attached to this cover letter is the initial summary paragraph of the proposed *Nature* article.

Sincerely,

Evan A. Perkowski

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

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