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 CO2 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 CO2 concentrations. Models that include photosynthetic acclimation to elevated CO2 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 CO2 are driven by changes in leaf nitrogen demand to build and maintain photosynthetic enzymes, which optimizes resource allocation to photosynthetic capacity and maximizes allocation to growth. 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 land surface models.

*Glycine max* L. (Merr) seedlings were grown under two CO2 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 growth chamber experiment. After seven weeks of vegetative growth, net photosynthesis, maximum rates of Rubisco carboxylation and RuBP regeneration, total leaf area, and whole-plant biomass were measured. We also calculated structural carbon costs to acquire nitrogen and plant investment to symbiotic nitrogen fixation.

Elevated CO2 increased net photosynthesis rates despite decreased maximum rates of Rubisco carboxylation and electron transport for RuBP regeneration. Elevated CO2 decreased the maximum rate of Rubisco carboxylation more strongly than it decreased the maximum rate of electron transport for RuBP regeneration, allowing net photosynthesis rates to be achieved through increasingly optimal co-limitation of Rubisco-limited and RuBP-limited photosynthesis rates. Leaf photosynthetic responses to CO2 were independent of fertilization or inoculation treatment, suggesting that leaf photosynthetic responses to elevated CO2 were driven by altered demand to build and maintain photosynthetic enzymes. On the other hand, increasing soil nitrogen fertilization enhanced positive effects of elevated CO2 on total leaf area and total biomass, responses that were associated with enhanced nitrogen uptake efficiency. Inoculation had no effect on whole-plant responses to elevated CO2 due to similar plant investments toward symbiotic nitrogen fixation between CO2 treatments.

Our results provide support for both the progressive nitrogen limitation and optimal resource allocation hypotheses, suggesting that each hypothesis operates at a different scale. Specifically, leaf photosynthetic responses to elevated CO2 were driven by changes in leaf nitrogen demand to build and maintain photosynthetic enzymes, supporting the optimal resource allocation hypothesis, while whole-plant responses to elevated CO2 were driven by changes in soil nitrogen supply, supporting the progressive nitrogen limitation hypothesis. These results also suggest that optimal resource allocation to photosynthetic capacity could result in nitrogen savings at the leaf level that may alleviate progressive nitrogen limitation at the whole-plant level.

We feel that this article would be of great interest to the broad readership of *Nature Communications*, as results 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.

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

Sincerely,

Evan A. Perkowski, Ph.D.

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

Dear Editorial Board at *Nature Communications*,

We are writing to submit our manuscript, titled “Nitrogen demand, supply, and acquisition strategy control plant responses to elevated CO2 at different scales”, for consideration as an article for publication in *Nature Communications*. The manuscript reports findings from an experiment that reconciles competing hypotheses for explaining the role of soil nitrogen availability on plant responses to elevated CO2, providing an avenue for improving future land surface model simulations.

In this study, we grew *Glycine max* L. (Merr.) seedlings under two CO2 concentrations, two symbiotic nitrogen-fixing bacteria inoculation treatments, and nine soil nitrogen fertilization treatments in a full-factorial growth chamber experiment. Elevated CO2 increased net photosynthesis rates despite reduced maximum rates of Ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) carboxylation and electron transport for RuBP regeneration. Leaf photosynthetic responses to elevated CO2 were independent of nitrogen fertilization and inoculation, and stronger

. Increasing nitrogen fertilization enhanced positive effects of elevated CO2 on total leaf area and biomass due to increased nitrogen uptake and reduced nitrogen acquisition costs. These results reconcile the role of nitrogen supply and demand on plant responses to elevated CO2, showing that leaf nitrogen demand to build and maintain photosynthetic enzymes drives leaf photosynthetic responses to elevated CO2, while nitrogen supply regulates whole-plant responses.