Dear Editorial Board at *AoB Plants*,

Please find our manuscript, titled *Symbiotic nitrogen fixation reduces carbon costs of nitrogen acquisition under low, but not high, nitrogen availability*, attached. The manuscript contains one table and three figures. We also include a supplementary materials document with three tables and one figure.

The purpose of the experiment was to understand interactive effects of soil nitrogen availability and symbiotic nitrogen fixation on plant carbon costs to acquire nitrogen. A previous study published by our group indicated that carbon costs to acquire nitrogen decreased with increasing soil nitrogen availability and that these responses were weaker in a species that form associations with nitrogen-fixing bacteria (Perkowski et al. 2021). These patterns were driven by reduced carbon costs to acquire nitrogen in the nitrogen-fixing species under low fertilization that diminished with increasing fertilization as investment in symbiotic nitrogen fixation decreased. While we speculated that this result might be due to a shift away from nitrogen uptake through symbiotic nitrogen fixation and toward direct uptake pathways with increasing fertilization, the study used species that confounded ability to acquire nitrogen through symbiotic nitrogen fixation with differences in phylogeny, life form, and growth duration, limiting our ability to provide a causal explanation for the different carbon cost to acquire nitrogen responses to soil nitrogen availability between the two species.

Here, we conducted a nitrogen fertilization-by-inoculation greenhouse experiment using soybean (*Glycine max* L. (Merr)) seedlings. Results indicate that inoculation with symbiotic nitrogen-fixing bacteria reduced carbon costs to acquire nitrogen, but only under low soil nitrogen fertilization where individuals invested more strongly in symbiotic nitrogen fixation. There was no inoculation effect under high soil nitrogen fertilization, presumably due to a shift away from nitrogen uptake through symbiotic nitrogen fixation and toward direct uptake as costs to acquire nitrogen through direct uptake became less than that of symbiotic nitrogen fixation. Nitrogen fertilization decreased carbon costs to acquire nitrogen. This study builds on findings from Perkowski et al. (2021), showing similar patterns while controlling for phylogeny, life form, and growth duration.

Plant nitrogen acquisition is a process in terrestrial ecosystems that links carbon and nitrogen cycles. Plants cannot acquire nitrogen without first allocating carbon belowground, which implies that there is an inherent carbon cost to the plant for acquiring nitrogen. Variation in the cost to acquire nitrogen may help explain the prevalence of different nitrogen acquisition strategies in different environments, but these costs have not been well quantified despite their inclusion in nitrogen uptake models currently implemented in terrestrial biosphere models. This work can be used to benchmark such nitrogen uptake models and refine the method in which biological nitrogen fixation is implemented in future versions of terrestrial biosphere models.

If you have any questions or concerns about our submission, please contact me at the e-mail listed below.

Sincerely,

Evan A. Perkowski (on behalf of co-authors Joseph Terrones, Hannah German, and Nick Smith)
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References

Perkowski EA, Waring EF, Smith NG. 2021. Root mass carbon costs to acquire nitrogen are determined by nitrogen and light availability in two species with different nitrogen acquisition strategies. Journal of Experimental Botany **72**, 5766–5776.