Dear Editorial Board at *Journal of Experimental Botany*,

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.

This manuscript details results from a greenhouse experiment where soybean (*Glycine max* L. (Merr)) seedlings were grown under full factorial combinations of nitrogen fertilization (two levels) and nitrogen-fixing bacteria inoculation (two levels) treatments. 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, published in *JXB*). 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 show similar patterns as observed in the previous study: increasing soil nitrogen availability decreased carbon costs to acquire nitrogen, a pattern that was stronger in uninoculated *G. max* than inoculated *G. max*. This result was driven by reduced carbon costs to acquire nitrogen in inoculated *G. max* under low soil nitrogen availability and similar costs between inoculation treatments under high soil nitrogen availability, suggesting that weaker carbon cost to acquire nitrogen responses to increasing soil nitrogen availability in plants inoculated with symbiotic nitrogen-fixing bacteria were likely driven by a shift away from nitrogen uptake through symbiotic nitrogen fixation and toward direct uptake pathways with increasing fertilization. This study builds on findings from Perkowski et al. (2021), showing similar patterns while controlling for phylogeny, life form, and growth duration.

Please note that this experiment is a direct follow up to two previous articles already published at *Journal of Experimental Botany* (Perkowski et al. 2021, Waring et al. 2023). Given this, we believe this manuscript is a good fit for the interdisciplinary audience at *Journal of Experimental Botany* and hope that the editorial staff will consider our paper as a full research article.

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)
Evan.a.perkowski@ttu.edu

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.

Waring EF, Perkowski EA, Smith NG. 2023. Soil nitrogen fertilization reduces relative leaf nitrogen allocation to photosynthesis. Journal of Experimental Botany **74**, 5166–5180.