Photosynthesis in terrestrial systems is constrained by ecosystem carbon and nutrient biogeochemical cycle dynamics (Hungate et al., 2003). Specifically, plants fix carbon dioxide from the atmosphere into simple sugars using enzymes, such as Ribulose-1,5-bisphosphate carboxylase/oxygenase (“Rubisco”), that have large nitrogen requirements to build and maintain (Evans, 1989). Recent photosynthetically derived carbon (“photosynthate”) can be accumulated as biomass (cite), lost as a substrate of plant respiration (Glover, 1973), or allocated belowground to acquire nutrients (cite). Belowground photosynthate can be used by plants to acquire nutrients either directly from the soil (cite), indirectly through root exudates that prime soil microbial communities and organic matter decomposition (Bengtson et al., 2012), or indirectly through symbioses with mycorrhizal fungi and/or symbiotic nitrogen-fixing bacteria (S. E. Smith & Read, 2008).

Anthropogenic activities have been the proximal cause of increasing atmospheric CO2 concentrations since the start of the Industrial Revolution in the mid 1700s. The Intergovernmental Panel on Climate Change suggests that atmospheric CO2 concentrations will continue to increase under business-as-normal emissions scenarios, with some scenarios suggesting that CO2 concentrations will exceed 1,000 ppm by 2100 (IPCC, 2013). Plant ecologists and physiologists have been long interested in understanding long-term effects of elevated CO2 on plant photosynthetic processes, where large swaths of studies report that increasing CO2 concentrations generally results in reductions in leaf nutrient allocation and photosynthetic capacity, a pattern that often corresponds with a stimulation in whole plant growth and net primary productivity (Ainsworth et al., 2002; Ainsworth & Rogers, 2007; Curtis, 1996; Makino, 2003; Morgan et al., 2004; Poorter et al., 2022; N. G. Smith & Dukes, 2013).

There are two conflicting hypotheses that explain the inverse leaf and whole plant acclimation responses to increasing CO2. Some have hypothesized that nutrient limitation may be the primary control of plant acclimation to CO2, as nutrient availability commonly limits primary productivity and may decrease over time in elevated CO2 environments (Fay et al., 2015; LeBauer & Treseder, 2008; Liang et al., 2016; Luo et al., 2004) through chronic stimulations in whole plant nutrient demand. The nutrient limitation hypothesis predicts that plants decrease leaf nutrient allocation and photosynthetic capacity as a direct response to progressive reductions in soil nutrient availability due to elevated CO2. The nutrient limitation hypothesis also predicts an acute stimulation in whole plant growth due to elevated CO2 that dampens over time because of progressive nutrient limitation.

An alternative hypothesis to the leaf response, based on photosynthetic least-cost theory (Prentice et al., 2014; Wright et al., 2003) suggests that plants growing under elevated CO2 environments instead downregulate nutrient allocation to Rubisco to optimize resource use efficiencies at the leaf level, which maximizes resource allocation to whole plant growth. Importantly, the nutrient limitation and least-cost hypotheses predict similar leaf acclimation responses to CO2, but result in different outcomes at the whole plant level.

Nutrient acquisition strategy, or the method in which plants acquire nutrients, may also impact how plants acclimate to CO2 (N. G. Smith & Keenan, 2020; Terrer et al., 2018). Plants acquire nutrients via direct uptake from their rooting systems or through symbiotic associations with mycorrhizal fungi or symbiotic nitrogen-fixing bacteria (S. E. Smith & Read, 2008). In plants that form associations with microbial symbionts, plants allocate recent photosynthate belowground in exchange for nutrients acquired by microbial symbionts. However, not all microbial symbioses require the same belowground carbon investments to exchange nutrients. Carbon costs to acquire nitrogen, or the amount of carbon plants allocate belowground per nitrogen acquired, vary across nutrient acquisition strategies and soil nutrient availability thresholds (Perkowski et al., 2021). Interestingly, a recent global meta-analysis indicates that carbon costs to acquire nitrogen may modify plant acclimation responses to CO2 (Terrer et al., 2016, 2018), although manipulation experiments that directly test the mechanisms driving these responses are rare.

In this study, I will investigate the influence of inoculation with symbiotic nitrogen-fixing bacteria and direct soil nutrient manipulation on soybean (*Glycine max* L.) acclimation responses to CO2. This experiment will determine whether nutrient limitation or optimal leaf resource investment is the primary driver of plant acclimation to CO2 and how nutrient acquisition strategy modifies these responses. I hypothesize that leaf acclimation to CO2 will be driven by optimal leaf resource investment, not nutrient limitation. Specifically, I predict that increasing CO2 will decrease stomatal conductance, leaf nutrient allocation, and photosynthesis independent of nutrient acquisition strategy or soil nutrient availability, which will maximize resource allocation to whole plant growth. While I do not expect that soil nutrients or acquisition strategy will modify leaf acclimation responses to CO2, I do expect that soil nutrient availability will increase the positive effect of CO2 on whole plant growth. I also predict that inoculation with nitrogen-fixing bacteria will increase whole plant growth responses to CO2. However, I only expect an inoculation effect in low soil nutrient environments, as inoculated individuals should shift away from nitrogen fixation and toward direct uptake with increasing soil nutrient availability (Perkowski et al., 2021; Rastetter et al., 2001).