**Methods**

*Experimental Design*

*Glycine max* were planted in 64 6-liter pots (NS-600, Nursery Supplies, Orange, CA, USA) containing unfertilized potting mix (Sungro Sunshine Mix #2, Agawam, MA, USA). Pots and potting mix were steam sterilized at XX C for XX hours prior to eliminate any bacterial or fungal growth. Thirty-two randomly selected pots were inoculated with *Bradyrhizobium japonicum* (Verdesian N-Dure™ Soybean, Cary, NC, USA) to stimulate root nodulation. The remaining 32 pots did not receive any inoculation treatment. Upon planting, pots were immediately placed in one of four random blocks in a greenhouse and received one of two nitrogen fertilization treatments as 150 mL of a modified Hoagland’s solution (Hoagland & Arnon, 1950) equivalent to either 70 or 630 ppm N twice per week for a span of six weeks. Nitrogen fertilization doses were received as topical agents to the soil surface and were modified to keep concentrations of other macro- and micronutrients equivalent (Supplemental Table S1). Throughout the experiment, plants were also routinely well-watered to eliminate any water stress potential.

*Leaf gas exchange and trait measurements*

Six weeks after experiment initiation, we sampled one random, fully expanded leaf with little to no visible external damage for gas exchange measurements. Leaves were attached to a Li-COR LI-6800 (Li-COR Bioscience, Lincoln, Nebraska, USA) portable photosynthesis machine to measure net photosynthesis (*A*net; μmol m-2 s-1), stomatal conductance (*g*s; mmol mol-1), and intercellular CO2 concentration (*C*i; µmol mol-1) at different atmospheric CO2 (*C*a; µmol mol-1) concentrations (i.e., an *A*net/*C*i curve). *A*net/*C*i curves were conducted under saturating light conditions (1,500 μmol m-2 s-1), 50% relative humidity, and cuvette temperature set to 25°C. We measured *A*area, *g*s, and *C*i at each of the following reference CO2 concentrations (*C*a; μmol mol-1): 400, 300, 200, 100 50, 400, 400, 600, 800, 1000, 1200, 1500, and 2000. We also collected chlorophyll fluorescence measurements for each focal leaf using a MultispeQ (PhotosynQ, East Lansing, MI, USA) device. Finally, we subjected individuals to at least a 30-minute period of no light and quantified dark respiration (*R*d; μmol m-2 s-1), again using a Li-COR LI-6800 under the same relative humidity and temperature settings as the *A*area/*C*i curve with incoming radiation set to 0 μmol m-2 s-1.

Leaf trait measurements were collected on the same focal leaf used to generate each CO2 response curve. Images of each leaf were curated using a flat-bed scanner to determine wet leaf area using the 'LeafArea' R package (Katabuchi, 2015), which automates leaf area calculations using ImageJ software (Schneider *et al.*, 2012). Each leaf was dried at 65C for at least 48 hours, weighed, and ground until homogenized. Specific leaf area (*SLA*; cm2 g-1) was calculated as the ratio of wet leaf area (*L*area) to dry leaf biomass (*L*mass). Using subsamples of ground and homogenized leaf biomass, we also determined leaf nitrogen content (*N*mass; g g-1) through elemental combustion analysis (Costech-4010, Costech, Inc., Valencia, CA, USA). Leaf nitrogen mass per unit leaf area (*N*area; g m-2) was then calculated by dividing *N*mass by *SLA*, then multiplying by 10,000 to convert cm-2 to m-2.

*Whole plant traits*

We harvested all experimental individuals and separated biomass of each experimental individual into major organ types (leaves, stems, roots) approximately seven weeks after experiment initiation. We also harvested root nodules when present. Leaf areas of all harvested leaves were measured using an XXX. Total leaf area (cm2) was calculated as the sum of all leaf areas, including the leaf area of the focal leaf measured during the CO2 response curve. All harvested material was dried in an oven set to 65°C for at least 48 hours, weighed, and ground to homogeneity. Total dry biomass was then calculated as the sum of dry leaf, stem, root, and root nodule biomass. We also quantified carbon and nitrogen content through elemental combustion (Costech-4010, Costech, Inc., Valencia, CA, USA) of each respective organ type using subsamples of ground and homogenized organ tissue.

We determined structural carbon costs to acquire nitrogen following the approach explained in Perkowski *et al.* (2021). Structural carbon costs to acquire nitrogen were calculated as the ratio of total belowground carbon biomass to whole plant nitrogen biomass. Belowground carbon biomass was calculated by multiplying the carbon content of roots and root nodules by total biomass of each respective organ type, then adding root carbon biomass and root nodule carbon biomass. Similarly, whole plant nitrogen biomass was calculated by multiplying the nitrogen content of leaves, stems, roots, and root nodules by biomass of each respective organ type, then taking the sum of nitrogen biomass of each organ type. Importantly, this calculation only quantifies plant structural carbon costs, and does not include any additional carbon costs associated with respiration, exudation, or turnover. An explicit explanation of the limitations of this calculation can be found in Perkowski *et al.*, (2021).

*Curve fitting and parameter estimation*

We fit *A*net/*C*i curves of each individual using the 'fitaci' function in the 'plantecophys' R package (Duursma, 2015). This function estimates the maximum rate of Rubisco carboxylation (*V*cmax; µmol m-2 s-1) and maximum rate of electron transport for RuBP regeneration (*J*max; µmol m-2 s-1) based on the Farquhar, von Caemmerer, and Berry biochemical model of C3 photosynthesis (Farquhar *et al.*, 1980). For each curve fit, we included triose phosphate utilization (TPU) limitation avoid underestimating *V*cmax and *J*max (Gregory *et al.*, 2021). We also determined kinetic parameters and CO2 compensation points using leaf temperature and equations derived in Medlyn *et al.* (2002). Dark respiration measurements were included in all curve fits and were first standardized to 25C using a log-polynomial approach explained in Heskel *et al.* (2016), where:

(Eqn. 3)

*R*T is the temperature standardized respiration rate, *T* is the temperature in which a given respiration rate is being standardized, set to 25C, and *T*ref is the temperature of the respiration measurement *R*Tref. *b* and *c* are coefficients that Heskel *et al.* (2016) derived from a log-polynomial approach described in O’Sullivan *et al.* (2013) for plant functional types and biomes. We used coefficients set by Heskel *et al.* (2016) for C3 herbaceous species, where *b* was set to 0.1271 and *c* was set to -0.00110.

For all *A*net/*C*i curve fits, we manually standardized *V*cmax and *J*max to25C using a modified Arrhenius equation (as in Kattge & Knorr, 2007):

(Eqn. 4)

where *k*25 represents the standardized *V*cmax or *J*max rate at 25C, *k*obs represents the *V*cmax or *J*max estimate at the average leaf temperature measured inside the cuvette during the CO2 response curve. *H*a is the activation energy of *V*cmax (71,513 J mol-1; Kattge & Knorr, 2007) or *J*max (49,884 J mol-1; Kattge & Knorr, 2007). *H*d represents the deactivation energy of both *V*cmax and *J*max (200,000 J mol-1; Medlyn *et al.*, 2002), and R represents the universal gas constant (8.314 J mol-1 K-1). *T*ref represents the standardized temperature of 298.15 K and *T*obs represents the mean leaf temperature (K) during each CO2 response curve. ΔS is an entropy term that Kattge & Knorr (2007) derived as a linear relationship with average growing season temperature (*T*g; °C), where:

(Eqn. 5)

and:

(Eqn. 6)

We estimated *T*g in equations 5 and 6 based on mean air temperature for each block throughout the duration of the experiment. These data were collected using HOBO MX2301 data loggers (Onset Computer Corp., Bourne, MA, USA), which recorded temperature and humidity of each block in the greenhouse on a fifteen-minute timestep. We then used *V*cmax25 and *J*max25 estimates to calculate the ratio of *J*max25 to *V*cmax25 (*J*max25:*V*cmax25) and the ratio of *R*d25 to *V*cmax25 (*R*d25: *V*cmax25).

*Statistical analyses*

We built a series of linear mixed-effects models to investigate the impacts of soil nitrogen fertilization and inoculation status on *G. max* leaf photosynthesis, tradeoffs between nitrogen and water use, and whole plant growth. All models included soil nitrogen fertilization, inoculation status, and interactions between soil nitrogen fertilization and inoculation status as categorical fixed effects. Block number was included as a random intercept term to account for any environmental heterogeneity within the greenhouse. Models with this independent variable structure were constructed to quantify relationships between soil nitrogen fertilization and inoculation status on *A*net, *V*cmax25, *J*max25, *J*max25:*V*cmax25, *R*d25, *R*d25:*V*cmax25, *N*area, *SLA*, *N*mass, *L*area, *L*mass, *g*s, χ, *PNUE*, *iWUE*, *V*cmax:*g*s, total biomass, and total leaf area.

We used Shapiro-Wilk tests of normality to determine whether linear mixed-effects models satisfied residual normality assumptions. All models satisfied residual normality assumptions except XXX, XXX, and XXX (Shapiro-Wilk: p<0.05 in all cases). We attempted to satisfy residual normality assumptions for these dependent variables by first fitting models using dependent variables that were natural log transformed. If residual normality assumptions were still not met (Shapiro-Wilk: p<0.05), then models were fit using dependent variables that were square root transformed. All residual normality assumptions were met with either a natural log or square root data transformation (Shapiro-Wilk: p>0.05 in all cases). Specifically, we natural log transformed XXX and XXX. We also square root transformed XXX.

In all statistical models, we used the 'lmer' function in the 'lme4' R package (Bates *et al.*, 2015) to fit each model and the 'Anova' function in the 'car' R package (Fox & Weisberg, 2019) to calculate Type II Wald's χ2 and determine the significance (α=0.05) of each fixed effect coefficient. Finally, we used the 'emmeans' R package (Lenth, 2019) to conduct post-hoc comparisons using Tukey's tests, where degrees of freedom were approximated using the Kenward-Roger approach (Kenward & Roger, 1997). All analyses and plots were conducted in R version 4.1.1 (R Core Team, 2021).

**Results**