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\subsection{Carbon costs to acquire nitrogen are determined by light and nitrogen availability}

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\noindent In the first experimental chapter, I quantified carbon costs to acquire nitrogen in a species capable of forming associations with symbiotic nitrogen-fixing bacteria (\textit{*Glycine max*}) and a species not capable of forming such associations (\textit{*Gossypium hirsutum*}) grown under four soil nitrogen fertilization treatments and four light availability treatments in a full factorial greenhouse experiment. I hypothesized that increasing light availability would increase carbon costs to acquire nitrogen due to an increase in plant nutrient demand, a pattern that would be driven by a larger increase in belowground carbon biomass than whole plant nitrogen biomass and would be apparent in both species. I also hypothesized that increasing fertilization would decrease carbon costs to acquire nitrogen due to an increase in plant nutrient supply, a pattern that would be indexed by a larger increase in whole plant nitrogen biomass than belowground carbon biomass with increasing fertilization. I expected that the negative effect of increasing fertilization on carbon costs to acquire nitrogen would only be apparent in \textit{*G. hirsutum*}, as \textit{*G. max*} would be able to acquire additional nitrogen needed to satisfy plant nitrogen demand through nitrogen fixation.

Supporting my first hypothesis, I found that increasing light availability increased carbon costs to acquire nitrogen in both species due to a larger increase in belowground carbon biomass than whole plant nitrogen biomass. These patterns were observed in both species. In support of my second hypothesis, I found that increasing fertilization decreased carbon costs to acquire nitrogen due to a larger increase in whole plant nitrogen biomass than belowground carbon biomass. While these patterns were observed in both species, carbon costs to acquire nitrogen in \textit{*G. max*} were less responsive to increasing fertilization than \textit{*G. hirsutum*}, providing some support for my second hypothesis. Root nodulation data indicated that \textit{*G. max*} shifted relative carbon allocation from nitrogen fixation to direct uptake with increasing fertilization, which may explain the reduced responsiveness of \textit{*G. max*} carbon costs to acquire nitrogen across the fertilization gradient.

Despite evidence that reductions in the response of \textit{*G. max*} carbon costs to acquire nitrogen to increasing fertilization may have been driven by shifts away from nitrogen fixation with increasing fertilization, I urge caution in assigning causality to these results. This is because \textit{*G. max*} and \textit{*G. hirsutum*} are not phylogenetically related and have different life histories. Specifically,\textit{*G. max*} is a herbaceous annual species, while \textit{*G. hirsutum*} is a woody perennial species. Differences in life history between the two species limit my ability to assess whether reductions in the negative effect of increasing fertilization on carbon costs to acquire nitrogen in \textit{*G. max*} were driven by shifts to direct uptake with increasing fertilization. However, these patterns were later confirmed in the fourth experimental chapter, where I quantify similar weaker negative effects of increasing fertilization on carbon costs to acquire nitrogen in \textit{*G. max*} that were inoculated with symbiotic nitrogen-fixing bacteria compared to \textit{*G. max*} that were left uninoculated across a similar soil nitrogen fertilization gradient.

Overall, results from this experiment provide strong support for hypotheses, showing that carbon costs to acquire nitrogen are dynamic across changing aboveground and belowground growing conditions. These patterns provide some evidence suggesting that summed costs of resource use expected from photosynthetic least-cost theory may be context dependent on growing environments, and could scale to influence leaf and whole plant acclimation responses across environmental gradients. Though not measured here, results from this experiment provided important context for patterns observed in subsequent chapters.

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\subsection{Soil nitrogen availability modifies nitrogen-water use tradeoffs in a mature temperate deciduous forest}

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\noindent In the second experimental chapter, I quantified a series of leaf traits to assess whether changes in soil nitrogen availability or soil pH drove changes in nitrogen-water use tradeoffs predicted by photosynthetic least-cost theory. I measured leaf traits of mature upper canopy deciduous trees growing in a nine-year nitrogen-by-sulfur field manipulation experiment, where experimental sulfur additions were added with intent to acidify plots. Following patterns expected from the theory, I hypothesized that increasing soil nitrogen availability would allow plants to create more photosynthetic enzymes per leaf, allowing similar photosynthetic rates to be achieved with lower leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$, increased leaf nitrogen content allocated to photosynthetic leaf tissue, and increased photosynthetic capacity. Following patterns observed in the light-by-nitrogen greenhouse experiment, I expected that this response would be driven by a reduction in the cost of acquiring nitrogen, which would cause trees to sacrifice inefficient nitrogen use to enable more efficient use of other resources that limit leaf photosynthesis (e.g., water). As increasing soil pH is generally associated with increasing soil nitrogen availability, I expected similar leaf responses to increasing soil pH.

In broad support of patterns expected from theory and my hypothesis, increasing soil nitrogen availability was associated with increased leaf nitrogen content, but not net photosynthesis, resulting in an increase in photosynthetic nitrogen use efficiency. In further support of theory, increasing soil nitrogen availability exhibited slight, but nonsignificant, decreases in leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$ and increases in measures of photosynthetic capacity. Perhaps the strongest evidence for the theory was a strong negative relationship between leaf nitrogen content and leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$, of which increased with increasing soil nitrogen availability through a stronger increase in leaf nitrogen content than leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$.

Interestingly, I find no effect of soil pH on nitrogen-water use tradeoffs aside from a marginal reduction in net photosynthesis rates that marginally reduced photosynthetic nitrogen use efficiency with increasing soil pH. Directionally, reductions in photosynthetic nitrogen use efficiency with increasing soil pH are expected per theory; however, this was driven by no change in leaf nitrogen content and a reduction in net photosynthesis. Theory predicts that these tradeoffs should be driven by no change in net photosynthesis and an increase in leaf nitrogen content. Regardless, the general null leaf response to changing soil pH may have been due to experimental treatments directly increased soil nitrogen availability and affected soil pH in opposite patterns, suggesting that soil nitrogen availability may be more important in dictating nitrogen-water use tradeoffs than soil pH per se.

Overall, findings from this experiment show that nitrogen-water use tradeoffs are modified by soil nitrogen availability, showing support for patterns expected from theory. Though, these patterns negate previous correlational studies across environmental conditions that show soil pH as a dominant driver of nitrogen-water use tradeoffs \shortcite{Paillassa2020,Westerband2023}. Additionally, I find that leaf nitrogen content was generally more closely associated with photosynthetic traits than soil nitrogen availability. I speculate that this may be due to challenges associated with quantifying soil nitrogen availability in 160 m$^2$ plots. Alternatively, photosynthetic least-cost theory predicts that leaf nitrogen content may be a downstream product of leaf nitrogen demand to build and maintain photosynthetic machinery, which is more likely a product of aboveground climatic factors such as light availability, CO$\_2$ concentration, temperature, or vapor pressure deficit \shortcite{Smith2019,Paillassa2020,Peng2021,Westerband2023}. In this study, I was not able to assess whether aboveground climatic conditions modified nitrogen-water use tradeoffs due to the short distance between plots and across sites, and suggest that future studies conduct similar sampling approaches across diverse climatic gradients.

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\section{Variance in leaf nitrogen content across a precipitation and soil nitrogen availability gradient in Texan grasslands}

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\noindent In the third experimental chapter, I quantified variance in leaf nitrogen content across a precipitation and soil resource availability gradient in Texan grasslands. Specifically, I measured area-based leaf nitrogen content, components of area-based leaf nitrogen content (leaf mass per unit leaf area, leaf nitrogen per unit dry biomass), leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$, and the unit cost of acquiring nitrogen relative to water in 520 individuals comprising 57 species. Following patterns expected from photosynthetic least-cost theory, I hypothesized that increasing soil nitrogen availability and soil moisture would have respective negative and positive effects on the unit cost of acquiring nitrogen relative to water. I also hypothesized that leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$ would be negatively related to vapor pressure deficit and positively related to the unit cost of acquiring nitrogen relative to water. Finally, I hypothesized that area-based leaf nitrogen content would be driven through a negative relationship with leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$. I expected that variance in area-based leaf nitrogen content across the environmental gradient would be driven by a negative relationship between leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$ and leaf mass per area, following patterns expected from leaf economics spectra and tradeoffs between leaf productivity and leaf longevity \shortcite{Wright2004,Dong2020,Reich2014,Onoda2017}.

Analyses from this experiment indicate that variance in area-based leaf nitrogen content was negatively associated with increasing soil nitrogen availability, soil moisture, vapor pressure deficit, and was negatively related to increasing leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$. Following patterns expected from theory, a path analysis revealed that the positive soil nitrogen-leaf nitrogen relationship was driven by a positive relationship between soil nitrogen availability and the unit cost of acquiring and using nitrogen relative to water, a positive relationship between the unit cost of acquiring and using nitrogen relative to water, and negative relationship between leaf $C\_\mathrm{i}$:$C\_\mathrm{a}$ and leaf mass per unit leaf area. Interestingly, there was no effect of $C\_\mathrm{i}$:$C\_\mathrm{a}$ on leaf nitrogen content per unit dry biomass, indicating that variance in area-based leaf nitrogen content across the environmental gradient was driven by a change in leaf morphology and not leaf chemistry.

Together, results from this experiment provide strong support for the integrated role of soil nitrogen availability and aboveground climate in determining leaf nitrogen content. Additionally, findings reported in this chapter provide additional context to understanding general soil nitrogen-leaf nitrogen-photosynthesis relationships, showing that variance in leaf nitrogen content due to increasing soil nitrogen availability is driven by a shift in the cost of acquiring nitrogen relative to water. This study is the first to directly quantify these patterns, as no previous study has investigated variance in leaf nitrogen content across soil nitrogen availability gradients while also quantifying the unit cost of nitrogen acquisition relative to water. Additionally, findings from this experiment indicate a high degree of variance in the unit cost of nitrogen acquisition relative to water across the environment, highlighting a future need in photosynthetic least-cost model development to consider dynamic implementations of this parameterized value in future model versions.

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\subsection{Optimal resource investment to photosynthetic capacity maximizes nutrient allocation to whole plant growth under elevated CO$\_2$}

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\noindent In the fourth and final experimental chapter, I quantified leaf and whole plant acclimation responses in \textit{*G. max*} grown under two atmospheric CO$\_2$ levels, with and without inoculation with \textit{*Bradyrhizobium japonicum*}, and across nine nitrogen fertilization treatments in a full factorial growth chamber experiment. Following optimal coordination and photosynthetic least-cost expectations, I hypothesized that leaves would acclimate to elevated CO$\_2$ by more strongly downregulating $V\_\mathrm{cmax}$ compared to $J\_\mathrm{max}$, which would allow net photosynthesis rates to be achieved through equal co-limitation of Rubisco carboxylation and RuBP regeneration. I also hypothesized this physiological response to correspond with a stronger downregulation in leaf nitrogen content than $V\_\mathrm{cmax}$ and $J\_\mathrm{max}$, which would increase nitrogen use efficiency through an increase in the fraction of leaf nitrogen content allocated to photosynthesis. At the whole plant level, I hypothesized that whole plant growth and total leaf area would increase under elevated CO$\_2$. Following theory, I predicted that leaf acclimation responses would be independent of soil nitrogen fertilization or inoculation status, but expected that increasing soil nitrogen fertilization would increase the positive effect of elevated CO$\_2$ through a reduction in the cost of acquiring nitrogen.

I found strong evidence that leaf nitrogen content, $V\_\mathrm{cmax}$, and $J\_\mathrm{max}$ were each downregulated under elevated CO$\_2$. A stronger downregulation in $V\_\mathrm{cmax}$ than $J\_\mathrm{max}$ and stronger downregulation in leaf nitrogen content than $V\_\mathrm{cmax}$ or $J\_\mathrm{max}$ provided strong support suggesting that leaves were acclimating to elevated CO$\_2$ by optimizing leaf photosynthetic resource use efficiency to achieve optimal coordination. In striking support of my hypotheses, I find strong evidence suggesting that leaf acclimation responses to elevated CO$\_2$ were decoupled from soil nitrogen fertilization and inoculation treatment, despite apparent strong increases in leaf nitrogen content, $V\_\mathrm{cmax}$, and $J\_\mathrm{max}$ with increasing fertilization and in inoculated pots. These findings contrast the current formulation of photosynthetic processes in terrestrial biosphere models, where many models simulate downregulations in leaf nitrogen content under elevated CO$\_2$ schemes as a function of progressive nitrogen limitation.

While soil nitrogen fertilization or inoculation treatments had no effect on leaf acclimation responses to elevated CO$\_2$, I found that increasing soil nitrogen fertilization increased the positive effect of elevated CO$\_2$ on total biomass and total leaf area, a pattern that was likely driven by a reduction in costs of nitrogen acquisition with increasing fertilization that maximized nitrogen uptake efficiency. I also find strong positive effects of inoculation on whole plant growth under elevated CO$\_2$, though this response was only observed under the lower soil nitrogen fertilization thresholds. As with the first chapter, the dampened effect of inoculation on measures of whole plant growth were likely due to a reduction in nodulation with increasing fertilization.

\section{Synthesizing common patterns observed across experiments}

\subsection{The cost of acquiring and using nitrogen is dynamic and context dependent on aboveground and belowground growing conditions}

\noindent There are currently two iterations of optimality models that employ the use of patterns expected from photosynthetic least-cost theory, one for C$\_3$ species \shortcite{Wang2017,Smith2019,Stocker2020} and one more recently for C$\_4$ species \shortcite{Scott2022}. In both model variants, costs to acquire and use nitrogen relative to water are held constant using a global dataset of $\delta^{13}$C \shortcite{Cornwell2018}. The C$\_3$ optimality model initially assumed a constant cost to acquire and use nitrogen relative to water value of 240 \shortcite{Wang2017}, later corrected to 146 \shortcite{Stocker2020}, while the C$\_4$ optimality model assumes a constant value of 166 \shortcite{Scott2022}.

In the first and fourth experimental chapter, I show consistent evidence suggesting that costs to acquire and use nitrogen are context dependent on aboveground and belowground growing conditions. Specifically, I show that carbon costs to acquire nitrogen generally increase due to factors that influence leaf nutrient demand to build and maintain photosynthetic machinery, such as light availability or atmospheric CO$\_2$. Additionally, I show that carbon costs to acquire nitrogen generally decrease with increasing soil nitrogen availability in both the first and fourth experimental chapter, and that these responses are context dependent on species dominant mode of acquiring nitrogen. In both experimental chapters, costs of water acquisition and use are assumed to be constant, as pots were well watered and without soil water stress \shortcite{Stocker2020}. These findings suggest that increases in the cost of acquiring nitrogen with increasing light availability and CO$\_2$ concentration increased the cost of acquiring and using nitrogen relative to water, while reductions in the cost of acquiring nitrogen with increasing fertilization decreased the cost of acquiring nitrogen relative to water.

Theory predicts that changes in the cost of acquiring nitrogen relative to water should have predictable impacts on tradeoffs between leaf nitrogen and water use. Specifically, the theory predicts that a reduction in the cost of acquiring nitrogen relative to water should cause plants to increase leaf nitrogen content and decrease stomatal conductance, a pattern that allows species to maintain photosynthetic rates with less efficient nitrogen use and more efficient water use. These patterns were observed in both the first and fourth experiment, with data for the first experiment visualized in \textit{*Appendix A*}. In both experiments, reductions in carbon costs to acquire nitrogen relative to water with increasing fertilization decreased the fraction of leaf nitrogen allocated to photosynthesis, allowing net photosynthetic rates to move forward with enhanced photosynthetic capacity and strong tradeoffs between nitrogen and water use.

In the third experimental chapter, I again find strong evidence suggesting that costs of nitrogen acquisition and use are dynamic and context dependent on environmental growing conditions. I find that costs of nitrogen acquisition and use relative to water decrease with increasing soil nitrogen availability, which is consistent with inferred patterns observed in the first and fourth experiment. One key observation from the third experimental chapter was that the cost of nitrogen acquisition and use relative to water exhibited strong variance within individuals across sites.

Overall, patterns from the first, third, and fourth experiment demonstrate strong evidence that the unit cost of acquiring and using nitrogen relative to water is a dynamic calculation that changes within individuals at sites and across environmental gradients. Thus, findings from these experiments indicate that constant unit cost of acquiring and using nitrogen relative to water values in current iterations of optimality models may contribute to erroneous errors in model simulation. Furthermore, these findings provide strong and consistent evidence calling for models to implement frameworks for including unit costs of acquiring and using nitrogen dynamically across environmental gradients.

\subsection{Leaf nitrogen-water use tradeoffs vary across soil nitrogen availability gradients, but are context dependent on whether nitrogen is limiting}

\subsection{Species identity strongly determines the net effect of soil nitrogen availability and climate on leaf physiology}

\section{Future work}

\section{Ode to learning}

\noindent I conclude this dissertation by reflecting on my time as a Ph.D. student at Texas Tech University, specifically in the Department of Biological Sciences and under the mentorship of Dr. Nick Smith. I began this research in Fall 2018 with a brief background in