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Anthropogenic effects are pervasive, ignoring country and even conservation boundaries. Nitrogen deposition, a major component of global change, alters nutrient limitation with cascading consequences for biodiversity and ecosystem function. This project investigates the effect of nitrogen addition on belowground plant traits, which are underexplored due to the unique challenges of studying them, yet are critical for understanding important processes such as carbon sequestration. Specifically, I take advantage of three long-term and on-going nutrient addition experiments at the Konza Long-term Ecological Research Site in Manhattan, Kansas. Using these platforms, I explored how nitrogen addition affects belowground traits of five different plant species in the tallgrass prairie community over a six-week data collection period, sampling each species at its peak flowering time. Belowground traits were overwhelmingly not responsive to N additions as compared to aboveground. Individual trait responses were species specific, making generalities of N responses challenging. Aboveground and belowground were found to be correlated with strength of correlation increasing with N additions. Understanding the relationship between plant traits and certain variables like nitrogen addition will help improve our ability to predict future responses to global change drivers.

BELOWGROUND TRAITS LACK RESPONSE TO CHRONIC NITROGEN

ADDITION IN THE TALLGRASS PRAIRIE

by

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CHAPTER I: INTRODUCTION

1.1 Overview and Ecological Context

Over the last century, the annual rate of global nitrogen (N) deposition has doubled (Sutton et al. 2011) with serious implications for plant growth, community structure, and ecosystem processes. Nitrogen is a limiting nutrient for plants (Elser 2007) as the necessary proteins for growth and function are 16% N (Frink et al. 1999, Mariotti et al. 2008). Understanding how plant traits respond to N deposition is a critical piece in predicting the consequences of global change, as functional trait expression is the mechanism that links organismal response to ecosystem functions such as carbon sequestration (**Fig. 1**) at larger scales (Lavorel and Garnier 2002, Suding and Goldstein 2008). Global change can cause a shift in plant traits through two key pathways: individual change or community change. Likely both plasticity in traits at the species level or diversity of traits at the community level are simultaneously responding to global change and causing consequences for ecosystem processes (**Fig. 1**). Trait-based studies aim to utilize functional plant traits to simplify complex community dynamics into ecological strategies that have implications for function.

However, plant trait work is limited as methods, until recently, have largely neglected both intraspecific trait variation (occurring within a given species) and belowground trait responses (Bolnick et al. 2011, Klimešová et al. 2018, 2019). This occurred despite evidence suggesting that (1) global change alters the growth of individual plants including their traits extensively (Garbowski et al. 2021, Larson et al. 2020, Iversen and McCormack 2021) and that (2) belowground traits are likely to be critical for important ecosystem functions like carbon sequestration and the associated feedbacks to climate change (Bastos and Fleischer 2021). Particularly, a deeper understanding of the aboveground - belowground plant relationship

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(Garbowski et al. 2021) is needed with recent calls to "start digging in grassy…biomes",

highlighting the need for belowground studies in grasslands (Ottaviani et al. 2020).

Figure 1. Global Change Affects Ecosystem Processes via Two Plant Trait Pathways
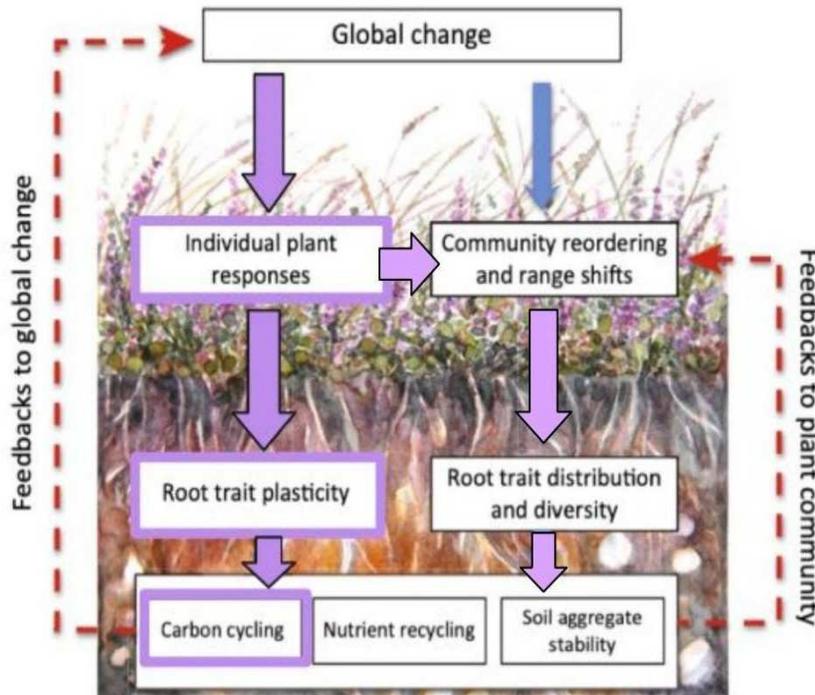
Note. First, global change can alter the community composition (right pathway). As

different species have different traits, changes in composition lead to changes in community

weighted traits. This avenue is commonly captured in global change studies and in ecosystem

modeling of function. The second is that intraspecific traits may vary in response to global

change (left pathway). My thesis focuses on this lesser studied pathway of individual plant

responses.

My research explores the effects of N addition on tallgrass prairie plant species traits. I

focused on the understudied components of (1) intraspecific trait responses and (2) belowground

dynamics. I studied five dominant (common and abundant) species in three independent long-

term nutrient addition experiments located at the Konza Prairie Biological Station in Manhattan,

Kansas. Aboveground trait measurements were taken in the field, then whole plant samples were collected and transported for *in-situ* processing of belowground traits and biomass weighing. **The overall aim of this research was to explore how intraspecific plant traits vary in response to long-term chronic nitrogen addition.** I achieved this through three targeted questions:

1. How do plant traits - in particular, root traits - change with nitrogen availability?
2. Does nitrogen availability influence the whole-plant niche space of a species and thus its functional role in the community?
3. Do belowground trait responses reflect tradeoffs between above and belowground traits?

1.2 Global Change: Nitrogen Deposition

Anthropogenic activities have led to profound alterations to the global N and carbon cycles (Elser 2007, Stevens et al. 2015) with agriculture being the largest culprit of human caused N pollution (Fields 2004, Fowler et al. 2013). Nitrogen enrichment on the global scale causes significant changes in plant productivity, plant community structure, and biodiversity (Stevens et al. 2015). In addition, rates of soil carbon sequestration, the process where plants convert atmospheric carbon dioxide into soil carbon (Yang et al. 2019), are positively correlated with greater plant diversity and larger plant biomass. Nitrogen availability exerts strong control on carbon storage where biomass is limited by N (Ontl and Schulte 2012), which is nearly all terrestrial systems including grasslands. Plant traits are the link between individual plant changes and ecosystem function (Hanisch et al. 2020), especially with nutrient-use traits like root traits influencing carbon input quantity and sequestration efficiency in grasslands (Athole et al. 2016, Bardgett et al. 2014, Chapin 2003).

1.3 Carbon Sequestration and Grasslands

Grasslands cover about 30% of North America and approximately 40% of the world's terrestrial surface (Global Land Cover database 2000, Joint Research Centre 2003). This major biome supports a range of essential ecosystem services, such as wildlife habitat, hydrological buffering, soil stabilization, carbon storage, and forage production (Gibson 2009). Most carbon storage takes place underground (Zhang et al. 2011, Xiao et al. 2014) where grasses may allocate between 40% and 80% of their net primary production (NPP) to roots (Silver et al. 2010). This is in stark contrast to forested ecosystems where much of the carbon is stored in the aboveground plant tissue of trees (**Fig. 2**; Xu et al. 2018). This makes grasslands particularly effective at storing carbon in areas prone to drought and wildfire (Dass et al. 2018). The significantly greater amount of soil organic carbon compared to other ecosystems is due to the rooting characteristics of grassland vegetation. These systems are adapted to disturbances like grazing and frequent fire by developing deep root systems and underground storage organs allowing quick regrowth aboveground post disturbance (Bond and Midgley 2012).

Figure 2. Vegetation and Soil Organic Carbon in Grasslands Versus Forest Ecosystems in China
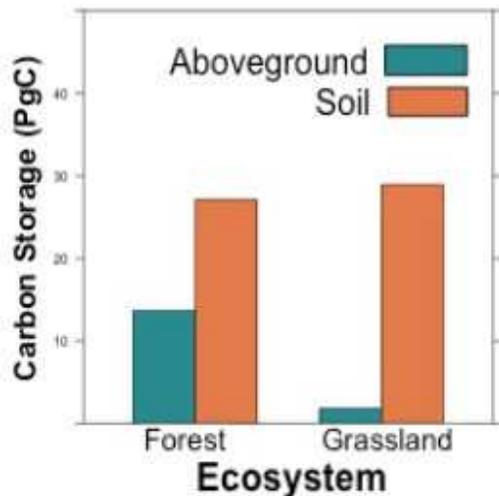
Note. Grasslands store carbon mostly belowground. Modified from Xu et al. 2018.

Occupying over 7 million km^2 of North American land, grasslands hold from 10 to 90 tons of carbon in the top 20 cm of topsoil per hectare (Silver et al. 2010), and are responsible for accounting for ~34% of all carbon sequestration in the U.S. Great Plains region (Pendell et al. 2018). Living biomass accounts for ~32% of carbon storage belowground, while soil organic matter accounts for 45% and dead biomass accounts for ~23% (Zu et al. 2012). These factors highlight the important role grasslands can play in carbon sequestration and therefore the global carbon cycle and the fight to mitigate global warming. Numerous grassland experiments have shown that plants with higher root mass tend to accumulate soil carbon at greater rates (Yang et al. 2019). Long-established grasslands like the tallgrass prairie ecoregion of the North American Great Plains can contain up to 10 tons of roots per acre with the top 24 inches of soil containing the vast majority (Williams 2001). Various studies of the potential for tallgrass prairie carbon storage have shown that carbon storage rates vary between 0.30 and 1.7 metric tons per acre per year (Williams 2001). This storage ability is cumulative over time, so these belowground plant

communities are able to sequester or store large volumes of carbon in a natural, safe, effective and reliable way. While the importance of belowground dynamics is well established, belowground dynamics are less frequently studied compared to aboveground dynamics including belowground plant traits (*e.g.*, root architecture, morphology, and nutrient acquisition) due to the challenging nature of such studies (Freschet et al. 2021). Critically, belowground responses must be incorporated into our understanding of how communities change if we are to understand global change impacts on ecosystem functions.

1.4 Plant Traits

The use of plant traits has been put forward as the holy grail mechanism to explain how changes at the organismal level lead to changes in ecosystem function (Lavorel and Garnier 2002, Suding and Goldstein 2008, Funk et al. 2017). For example, changes in root traits in response to N deposition could lead to changes in carbon sequestration with implications for global warming (Freschet et al. 2020). However, using functional trait responses to predict the effect of global change on ecosystem function currently has two key limitations. First, the majority of research focuses on interspecific variation, using the variation in continuous traits measured at one point in space and time (Petchey and Gaston 2006, Wright et al. 2006). While this approach works well for thinking about how traits change as communities change (**Fig. 1**, right pathway), it ignores the fact that intraspecific trait variation is common (**Fig. 3**; Henn et al. 2018) and an important component of trait-based processes (Albert et al. 2011, Yang et al. 2020). High variation in intraspecific traits demonstrates the importance of incorporation in trait studies. Second, trait-based studies tend to only measure aboveground traits due to their ease of collection and their importance in biomass production and net ecosystem exchange, but belowground traits are likely equally or even more critical for other important functions such as

carbon sequestration (Bardgett et al. 2014). This is particularly important in ecosystems such as grasslands where more than half of their biomass can be stored belowground (Titlyanova et al. 2009).

A gap exists between our understanding of plant traits and our understanding of whole-plant responses to nutrients, such as plant traits that describe how plant biomass is allocated belowground, including study of fine roots, coarse roots, and branching intensity (Mason et al. 2017, van der Plas et al. 2020). Plants invest in their organs according to an overarching trade-off between maximizing resource acquisition and productivity or maximizing resource conservation and longevity, called the "fast-slow" Resource Economics Spectrum (Wright et al. 2004, Reich 2014). This theoretical framework has been successfully linked to plant performance at the leaf level (Wright et al. 2004) but still remains contended when it is applied to roots (Freschet et al. 2021). Referred to as the Root Economics Spectrum, at one end of the spectrum plants with a "fast" belowground resource acquisition strategy construct long, narrow-diameter roots with minimal biomass investment but high metabolic rates (Reich 2014), while at the other end plants with a "slow" strategy achieve longer life span and prolonged return on investment by constructing thicker-diameter, denser roots (Bergman et al. 2020).

Figure 3. Variance Partitioning Analysis Demonstrating High Intraspecific Variation with Nitrogen-related Plant Traits: N:P, %N, C:N
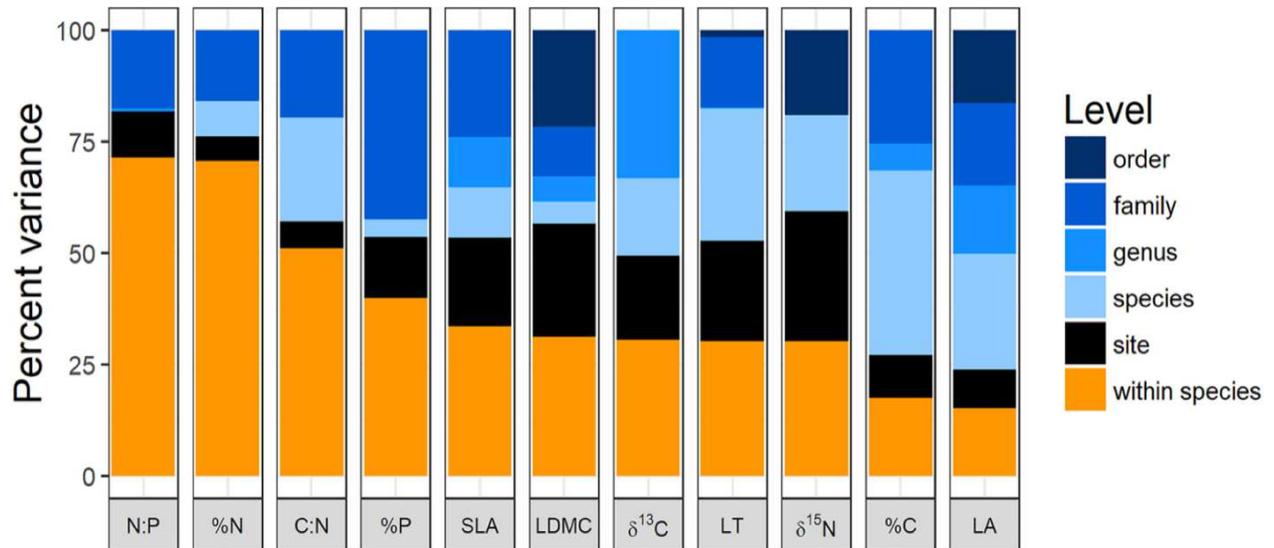
Note. Moderate intraspecific variation found in plant traits: (SLA) Leaf Area, (LT) Leaf Thickness. This suggests that intraspecific variation is common and an important source of variation warranting further study, particularly so for N related traits. Image Source: Henn et al. 2018.

It is clear that plants allocate biomass to different organs in response to nutrient variation in order to maximize production, yet a framework is lacking that adequately integrates plant responses with simultaneous variation in above- and belowground resources (Umaña et al. 2020). Characterizing a trait space that considers both above and belowground traits can expose species differentiation in ways that are not apparent in the individual spaces considering only aboveground (Reich 2014, Díaz et al. 2016) or fine-root traits (Bergmann et al. 2020), helping to better understand species coexistence and diversity patterns.

1.5 Objectives

My research uses a trait-based approach that incorporates intraspecific trait variation and belowground traits to explore how plant traits vary in response to long-term chronic N addition. Plant traits related to size and growth rate affect most key processes in the cycling of carbon and nutrients (Chapin 2003). Increases in aboveground plant productivity and corresponding aboveground plant traits have been well documented with N-addition; however, belowground traits are still largely understudied creating a large source of uncertainty in predicting carbon cycle responses (Norby and Luo 2004). Plant traits are typically assigned to species as fixed traits. However, many plants are plastic in their response to the environment. Occurring at both interspecific and intraspecific levels, trait plasticity has been shown to be an important mechanism for enabling plants to persist within communities and to better tolerate changing environmental conditions under climate change (Henn et al. 2018). The incorporation of belowground trait changes with altered resource availability and embracing intraspecific trait variation is necessary to better predict functional processes of grasslands with future global change. My aim was to understand how global change factors influence critical ecosystem processes such as net primary productivity and carbon sequestration through changes in traits of species. Specifically, my overall aim was to **understand how chronic nitrogen addition influences intraspecific trait dynamics.** While my overarching aim addresses both above and belowground traits, the belowground component is particularly novel, and I explored this with three targeted questions.

Question 1: How do plant traits - in particular, root traits - change with nitrogen availability? Native grassland plant species have extensive and deep root systems with most species' allocating more biomass belowground than aboveground (Poorter et al. 2012). Besides

providing anchorage, the primary function of roots is to take up growth-limiting resources from the soil. Plant root systems have evolved a wide variety of forms and functions contributing to nutrient allocation, storage, and nutrient acquisition (**Fig. 4**; Bardgett et al. 2014). Some roots like rhizomes act as long-term storage, whereas fine roots turn over frequently. My study site in the tallgrass prairie has both inter- and intraspecific diversity in root structure where the plant community comprises fine roots, coarse roots and storage roots. A more detailed understanding of biomass allocation in roots is needed to understand its role in carbon sequestration storage. As roots are difficult to study being hidden in the soil matrix, their response to nitrogen availability is less well studied compared to their aboveground counterparts. By examining root trait characteristics through measurements of root length, biomass, diameter size and nutrient content- I paint a picture of overall underground biomass allocation and how nitrogen deposition may shift that.

Question 2: Does nitrogen availability influence the whole-plant niche space of a species and thus its functional role in the community? A particularly important concept that unifies many ecological and evolutionary theories is the concept of the Hutchinsonian multidimensional niche (Hutchinson 1957). Functional diversity is also recognized as an important approach for understanding species coexistence and community assembly (Mouchet et al. 2010, Mason et al. 2013), while also characterizing the functional responses of plant communities (Lavorel 2013). The functional trait space concept, first proposed by Hutchinson (1957), is quantified based on trait multi-dimensional hypervolumes, which is characterized by phenotypic space occupied by individual plants of a species in a given environment. Quantifying the functional trait space at different N treatments across species enables inferences about how aspects of global change can alter functional diversity and ecological strategies.

Figure 4. Root Traits are Complex, Yet Can be Categorized into Four Main Groups
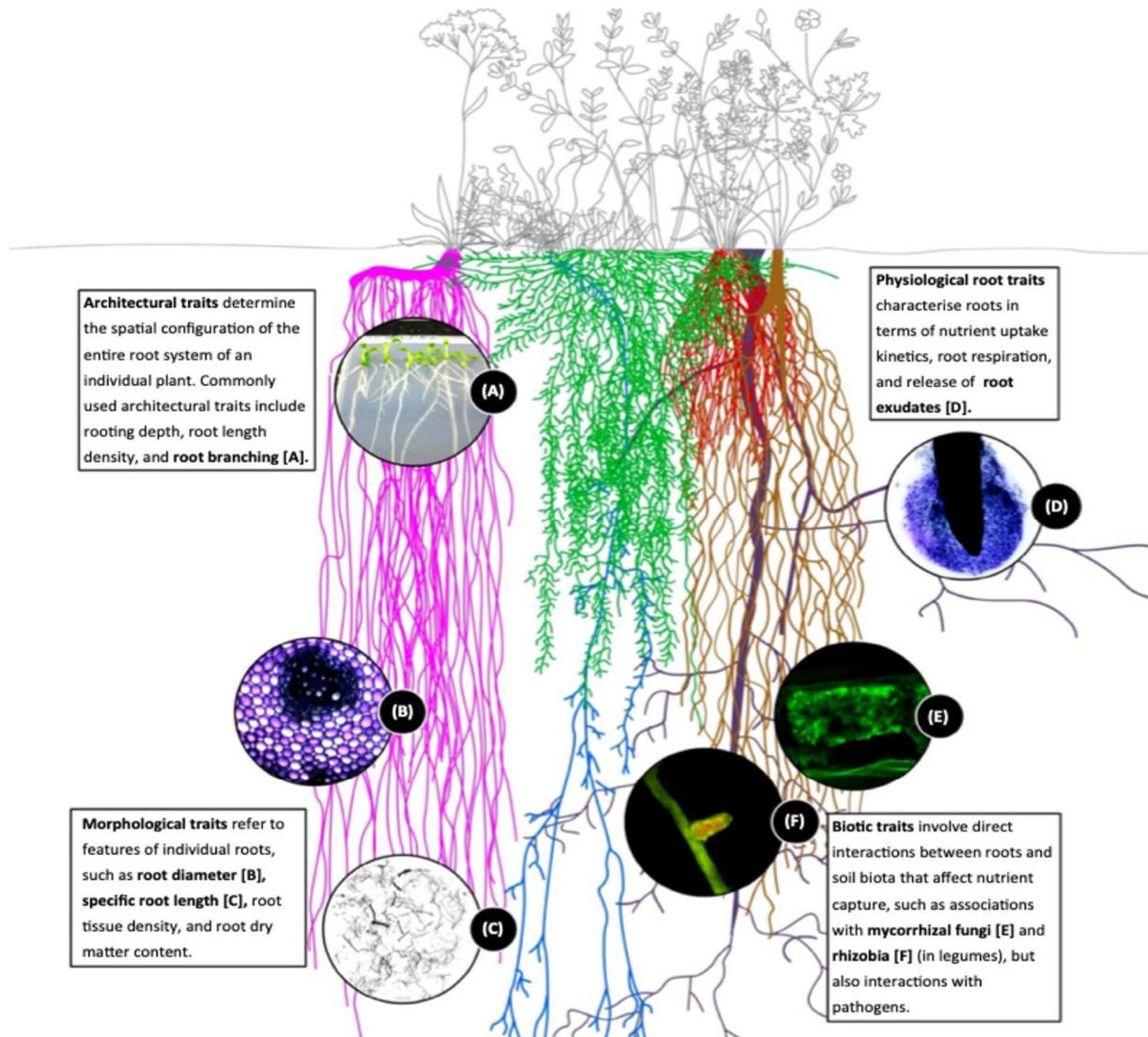
Note. Each trait can be studied independently or combined as a whole to understand the niche space a plant inhabits and thus its functional roll in the community. My data collection focused on architectural, morphological, and physiological traits. Image source: Bardgett et al. 2014.

Question 3: Do belowground trait responses reflect tradeoffs between above and belowground traits? If aboveground net primary production (ANPP) increases with nutrient

additions, does the corresponding trade-off mean that belowground net primary production (BNPP) decreases? Evolutionary adaptation to various ecological pressures may lead to plant phenotypic changes (Herms and Mattson 1992). As a result, general trade-offs exist across ecosystems. When resources are limited, plants have to choose where to allocate those resources. For the aboveground traits measured in my study, complimentary belowground traits were also collected with which to compare. Studying this trade-off has significant novel value where belowground traits are previously understudied due to the challenging nature in trait accessibility. If we can define commonly found trade-offs, we can infer belowground changes by measuring their aboveground counterparts.

2.1 Site Description and Experimental Set up

The fieldwork was conducted at three experimental sites within the Konza Prairie

Biological Station (KPBS), in Manhattan, Kansas. KPBS, part of the long term ecological

research (LTER) network, facilitates comprehensive ecological research focused on the tallgrass

prairie (**Fig. 5**), one of the most productive grasslands in North America (Knapp et al. 1998).

KPBS has an average aboveground net primary production (ANPP) of 354 ±135 g/m^2 with a

Mean Annual air Temperature (MAT) of 12.1°C and a Mean Annual Precipitation (MAP) of 826

±198 mm (Petrie et al. 2017). With 64.6% of its species classified as perennial grasses, the

composition of KPBS is over 90% native tallgrass prairie (Dong et al. 2012). Dominant C4

grasses of this site include *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum* and

Schizachyrium scoparium. Numerous sub-dominant grasses, forbs and woody species also

contribute to the prairie's high floristic diversity (Towne 2002).

Figure 5. Map of the USA Prairie Regions, Prior to Conservation
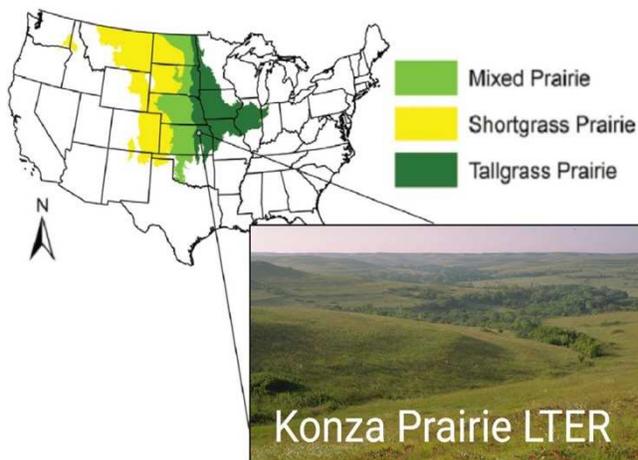
Note. Image source: The Nature Conservancy. Map modified from Vero et al. 2017 in

BioRender.

At KPBS, experiments are conducted at both the large scale with watershed-level treatments of fire and grazing, and at the small scale with treatment plots that allow manipulation of nutrients, plant species, or soil microorganisms. This site offers a uniquely rich facet of ecological research, building upon a legacy of these long-term studies to address the effects of global change. This study utilized 3 long term experimental platforms – ChANGE, NutNet, and P-Plots which have been adding N to upland, unplowed, native tallgrass prairie for 8, 15, and 19 years respectively (**Table 1**). By using multiple independent long term experimental nutrient additions, this increased replication and power to detect differences as well as maximize the ability to draw generalities from these results. Each of these experiments have many more treatments than what I used for my study. I focused on the N only addition plots. Nitrogen is added to the plots yearly in May as a fertilizer (slow-release urea for ChANGE and NutNet; ammonium nitrate for P-Plots). Species composition of the plant community is collected in June and August of each year, and a single weather station located nearby collects daily precipitation and temperatures.

Table 1. Long-term Experimental Platforms at the Konza Prairie LTER where Plant Traits were Collected on Five Abundant and Common Species

Experiment	N Treatment	Start Date of Treatments	Replication & Design	Number of Plants Measured & Harvested
ChANGE	1. 0 g N/m^2 (C) 2. 2.5 g N/m^2 3. 10 g N/m^2 4. 20 g N/m^2	2014	n = 6 in a block design	1 individual per plot X 6 replicate plots X 4 trts X 5 species = 120 plants
NutNet	1. 0 g N/m^2 (C) 2. 10 g N/m^2	2008	n = 3 in a block design	2 individuals per plot X 3 replicate plots X 2 trts X 5 species = 60 plants
P-Plots	1. 0 g N/m^2 (C) 2. 10 g N/m^2	2003	n = 6 randomly distributed trts throughout plot	1 individual per plot X 6 replicate plots X 2 trts X 5 species = 60 plants

2.2 Study Species

Above and belowground traits in five of the most common and abundant grasses and forbs were collected in summer 2021. Trait data from two plant species that flower in early season *(Dichanthelium oligosanthes, Ambrosia psilostachya)* were collected in June 2021, one species that flowers in mid-season (*Solidago missouriensis*) was collected in July 2021, and two that flower late-season (*Andropogon gerardii, Sorghastrum nutans*) were collected in August 2021 (**Fig. 6**). *A. gerardii* and *S. nutans* are tall, clonal, perennial, C4 grasses while *D. oligosanthes* is a short-statured, perennial C3 grass (Dong et al. 2012, Ott and Harnett 2012). The two focal forb species, *S. missouriensis* and *A. psilostachya* are clonal, herbaceous, C3 perennials (Dong et al. 2012, Preus and Morrow 1999). For each species, collection of aboveground plant measurements and specimens for 48 plants of a given species was done within a single week to avoid differences in intraspecific variation due to seasonality. A sum total of 240 plants (n = 240)

was sampled for this study.

Figure 6. Five Common and Abundant Species of the Tallgrass Prairie Sampled in Three Flowering Seasons

Note. Image Source: Konza Photo Galleries.

2.3 Trait Data Collection

I collected plant trait data in the field as well as in the lab (**Table 2)**, and all traits were collected on each plant individual allowing for the linkage of trait responses. After locating the individual in the field, I measured height, canopy width, number of leaves, then recorded leaf status as emerged, fully emerged or senescing. I tagged the focal plant at the base of the stem for ease of identification in later root washing. After cutting the aboveground portion of the plant at ground level, I put the sample in a Ziploc bag with a moist paper towel into a cooler. Collection of belowground plant samples was standardized by using a large soil corer (6.9 cm width) to a depth of 15 cm. Root samples were placed in a Ziploc bag and directly into a cooler. Above and belowground portions were transported back to the Konza Prairie Ecology Lab for processing.

In the lab, three fully emerged leaves were clipped for each plant. Using calipers, leaf thickness was recorded per leaf. The leaf was then weighed to get wet biomass and scanned for later calculations (**Appx. B**) of specific leaf area (SLA) in imaging software, ImageJ (Schneider et al. 2012). Each leaf was put into a labeled coin envelope and dried at 60°C for 2 days (48

hours) with the remainder of the aboveground biomass assigned to a labeled paper bag. The aboveground sample was weighed to get dry biomass with each of the three leaves weighed on a per leaf basis.

In the lab, the root samples were soaked in a water bucket to aid in loosening of the soil structure for later root washing. Root soak did not exceed 12 hours to prevent nutrient leaching of tissue samples (Freschet et al. 2020). Using a sieve, water bottles, and paintbrushes, the soil core sample was cleaned of soil and the tagged focal root was separated from the remainder of the belowground biomass in the soil core (**Fig. 7A**). The focal root was then put into a labeled paper bag and dried at 60°C for two days (48 hours) before weighing. The remainder of the belowground biomass in the sample was also put into a separate labeled paper bag to be dried and weighed with the combined focal root biomass and soil core biomass referred to as total root biomass (**Appx. B**).

Figure 7. Root Samples were Processed by (A) Root Wash and (B) Photographed for Later Trait Calculations in Imaging Software
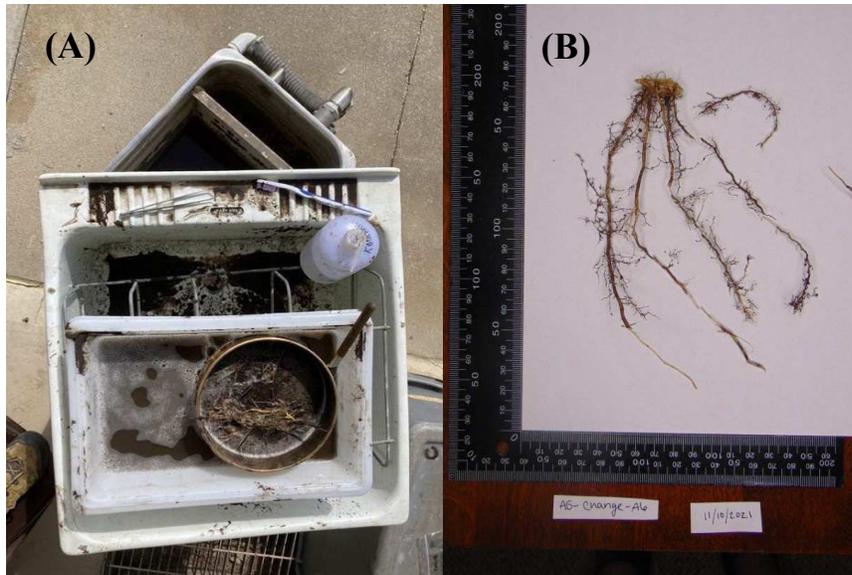
To ensure entire sample identification accuracy, the root samples went through a secondary round of scrutinous root sorting in the lab. Broken root pieces were matched and identified using a microscope. Roots were carefully laid out and photographed on a flat surface and specific root length (SRL) (**Appx. B**), maximum root diameter and mean root diameter were calculated using the Root Image Analysis (RIA) plugin through the Fiji platform for ImageJ software version 1.44 (Lobet et al. 2017, Schindelin et al. 2012; **Fig. 7B**). For every individual, a subsample of leaf and fine root material was crushed using an herb grinder and sent to Kansas State University lab for processing and analysis of leaf N and C content and root N and C content.

Table 2. Plant Traits (18) Collected Summer 2021 on 48 Individuals of Each of Five Species

Trait Category	Traits	How Measured
Aboveground	Plant Height	Measured in the field using a meter stick
Aboveground	Plant Volume	Measured in the field using a meter stick (Plant height x width1 x width2)
Aboveground	Leaf Number	Counted by hand in the field
Aboveground	Leaf Status*	Divided into stage of development (emerging, fully emerged, or senescing)
Aboveground	Leaf Thickness*	Measured using calipers
Aboveground	Specific Leaf Area (SLA)*	Single leaf taped on paper and scanned for area; Used scale to measure single leaf mass
Aboveground	Leaf %N*	Dried/crushed plant material sent to KSU for N content
Aboveground	Leaf %C*	Dried/crushed plant material sent to KSU for C content
Aboveground	Aboveground Biomass*	Use scale to measure total dried plant mass
Belowground	Specific Root Length (SRL)*	Root washed, photographed and put into ImageJ for length; Used scale to measure total dried root mass
Belowground	Focal Root Biomass*	Used scale to measure the mass of the dried focal root
Belowground	Total Root Biomass	Used scale to measure the mass of the total dried biomass of the core sample
Belowground	Mean Root Diameter*	Measured using root imaging software, Image J
Belowground	Maximum Root Diameter	Measured using root imaging software, Image J
Belowground	Root %N*	Dried/crushed plant material sent to KSU for N content
Belowground	Root %C*	Dried/crushed plant material sent to KSU for C content

Note. All traits in the table are used to answer my overarching aim. * indicates traits used in analysis (PCA) for question 2 and Pearson's correlation analysis for question 3.

2.4 Data Analysis

For all analyses, the data were subset into two different datasets: N addition and N gradient. For the N addition dataset, I used all data from the 0g and 10g N m^{-2} plots across all three experimental platforms (**Table 1**). For the N gradient dataset I only used data from the ChANGE platform which included 0g, 2.5g, 10g and 20g N m^{-2} plots (**Table 1**). For each analysis, separate models were run for each species so as to focus on intraspecific responses, rather than interspecific responses. Adjusted R^2 values were used and p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method (Benjamini and Hochberg 1995). When needed, appropriate *post-hoc* tests were used to identify significance among treatments. All statistical analyses were performed using R software 4.0.5 (R Core Team 2021).

Q. 1 – Trait response to N: For the N addition dataset, to determine the effect of N addition on traits I used mixed-model ANOVAs with nutrient treatment as a fixed effect and both experiment and block as random effects. As mentioned above, a separate ANOVA was run for each species but also for each trait. For the N gradient dataset, I regressed the trait versus N gradient to explore if the amount of N influences the pattern of trait response. Plot and block were included in the linear model (General Linear Model) as random factors. A separate linear model was run for each species as well as for each trait, adjusting p-values for multiple comparisons (Benjamini and Hochberg 1995). To create a visual summary of all the trait responses to N, I calculated the percent of traits which responded significantly for each species for all aboveground and for all belowground traits.

Q. 2 – To explore how traits are moving in trait space, I ran a principal component analysis (PCA) for both datasets, separately by species. First, a Bray-Curtis dissimilarity matrix was created for each species comparing all traits to each other and principal component analysis

(PCA) was used to visualize the variation across treatments. I focused on a subset of the 18 total collected traits, as some traits covaried (**Table 2**). A cutoff score of $r < 0.6$ was used to eliminate similar traits from the PCA analyses. R-values reported were averaged across species. Traits omitted from the PCA analyses were plant height, leaf number, number of fully emerged leaves, number of senescing leaves and maximum root diameter. Additionally, plant volume (**Appx. B**) was omitted because of the subjectivity of collection and total root biomass (**Appx. B**) was omitted as this incorporated biomass from other species, representing a "community" trait rather than a species trait. A suite of 11 traits (6 aboveground and 5 belowground) were kept in the PCA analysis: SLA, leaf thickness, number of fully emerged leaves, aboveground biomass, leaf %N, leaf %C, SRL, focal root biomass, root mean diameter, root %N and root %C.

The trait data was scaled using *prcomp* function (version 3.6.2) in R before performing a PCA using the *vegan* (version 2.4.2) Community Ecology package in R (Dixon 2003). Normalizing the data was necessary so that all variables have a zero mean and have the same standard deviation, thus all traits have the same weight and the PCA calculates relevant axes. The centroid of each treatment group was depicted using PCA, with a separate analysis run for each species in the two datasets. A PCA was used because it is a useful tool in combining information from multiple dimensions into a more palatable graphical visualization with two axes.

Multiple metrics exploring differences in trait space due to N treatment were explored. Permutational multivariate analysis of variance or PERMANOVA (999 permutations, *Adonis* function) was used on each species in each datasets to test the hypothesis that the centroids of individual trait responses varied by treatments (Anderson 2017). *Post-hoc* tests for multilevel pairwise comparisons (*pairwise.adonis* function) were used to determine significance among

treatment groups in the N gradient dataset (Martinez 2020). The distances between centroids (dissimilarity among treatments) were then calculated using a betadisper test in the *vegan* package. Betadisper is an implementation of Marti Anderson's Permdisp method, a multivariate analogue of Levene's test for homogeneity of variances (Anderson 2006, Anderson et al. 2006). *Post-hoc* tests of Tukey's Honest Significant Differences (TukeyHSD.betadisper) were used to identify significance between treatment groups in the N gradient dataset. Statistical test of differences in function diversity incorporating multiple traits, functional diversity (FDis), was calculated (**Appx. C**) by measuring the mean distance in multidimensional trait space of individuals to the centroid of all treatment groups (Laliberté and Legendre 2010). The *fundiversity* and *FD* packages in R were used to calculate functional trait diversity indices (Grenié and Gruson 2021, Laliberté and Legendre 2010) across species (de Bello et al. 2013).

The average values of the trait data were scaled to a mean of 0 so that each trait had the same weight in functional diversity measures and the units of trait values had no influence. A Gower dissimilarity matrix (*gow.dis* function) was used to calculate pairwise dissimilarities or the distance between traits (Gower and Digby 1981). Commonly, the calculation of functional dispersion refers to the mean distance of individual species to the centroid of all species in multidimensional trait space, weighted by species relative abundance. For my analyses, FDis is calculated separately for each species using the mean distance of individuals to the weighted centroid of all treatments in trait space (Laliberté and Legendre 2010). Commonly, measurements of FDis are weighted using the relative abundances of the species, where my data has equal abundance of individuals across treatment groups and is unweighted. Next, I calculated a related metric, functional richness (FRic), defined as the amount of niche space occupied by a species in a community. In this study, FRic is the volume of the convex hull in multidimensional

space, representing the total volume of trait space occupied by a N treatment group for a species. Computation of functional richness for 2.5g and 20g N m^{-2} plots was not possible with formula limitations in the aggregate number of traits exceeding the sample number per species (**Appx. C**). Measurements of FRic are calculated by treatment for each species, using code (**Appx. C**) modified from Grenié and Gruson (2021) examples using the *fd_fric* function in the *fundiversity* (version 0.2.1.9) package (Grenié and Gruson 2021).

    Q. 3 – I approached the trade-offs analysis using Pearson's correlation analysis (Freedman et al. 2007). In order to determine the relationship between the above- and belowground traits of the five species in different treatments and if it differed, I used the *ggpairs* function (Emerson et al. 2012) in the *GGally* R package (Schloerke et al. 2020) for plotting and calculated the correlation and significance between each trait pair based on Pearson's correlation analysis. A correlogram or generalized pairs plot for each species was used to visualize the relationship between each pair of above- and belowground traits, depicting trait distribution with scatterplots and trait relationships with linear regressions across treatments. Correlation statistics calculated within the plotting function were Pearson's product moment correlation, r and the significance of the least squares regression, p-values (adjusted for multiple comparisons using Benjamini-Hochberg method). The subset of 11 total traits used for the PCA were used for correlation matrix analyses for accuracy of pairwise trait comparison across treatments. Only above- and belowground trait pairs (**Appx. A1-5**, pink shaded plots) were used from the correlograms, all other trait pairs (above- and above-ground trait pairs or below and belowground trait pairs) regardless of significance were not relevant to the research objective for this particular study. High correlation reported relates to an r-value between 0.6 - 0.79 and an r-value reported between 0.8 - 1.00 shows very high correlation (Chan 2003, Quinnipiac University).

CHAPTER III: RESULTS

3.1 Q1: Trait Response and Relationship to Nitrogen

Overall, species show wide variation in trait response to chronic nitrogen (**Table 3-6**). For the N addition dataset (**Table 3-4**), each aboveground trait was significantly impacted by N addition in at least one species except for plant height which was not significantly affected by N addition for any species (**Table 3**). %N in the leaf was significant in 3 out of 5 species making it the most responsive trait. No trait was significant in all species. *D. oligosanthes* was the most responsive species in aboveground traits with 8 out of 11 (72.7%) aboveground traits statistically significant (**Table 3**). *Ambrosia psilostachya* was the second most responsive species, with 5 of 11 (45.5%) aboveground traits statistically significant. The remaining study species: *S. missouriensis*, *A. gerardii* and *S. nutans* each only had one aboveground trait that was statistically significant (**Table 3**). In addition to significance of response, magnitude of response varied across species with *A. psilostachya* typically showing the greatest magnitude of response when a trait was significant. In contrast to aboveground traits, the functional traits belowground lacked responsiveness to 10g N for all species (**Table 4**). Out of 7 belowground traits, root %N was the only trait with significant change, increasing with N addition in all five species (**Table 4**). All significant aboveground and belowground trait responses for all species were all increases in response to N addition (**Table 3-4**).

Table 3. Means and Statistical Results from Mixed-model ANOVAs Comparing Aboveground Traits in Control vs 10g m⁻² N

Trait	Treatment N m⁻²	*D. oligosanthes* Means	F-Statistic (p-value)	Relationship Direction	*A. psilostachya* Means	F-Statistic (p-value)	Relationship Direction	*S. missouriensis* Means	F-Statistic (p-value)	Relationship Direction	*A. gerardii* Means	F-Statistic (p-value)	Relationship Direction	*S. nutans* Means	F-Statistic (p-value)	Relationship Direction
Leaf Number	0N	**7.437**	**12.37 (<0.001)**	↑	**14.730**	**5.221 (0.029)**	↑	23.140	0.008 (0.929)		12.940	0.830 (0.369)		8.750	0.302 (0.587)	
	10N	**13.560**			**23.280**			22.820			14.830			9.500		
Leaf Thickness (cm)	0N	0.013	0.407 (0.528)		**0.032**	**5.128 (0.031)**	↑	0.046	3.627 (0.067)		0.041	0.666 (0.422)		0.031	2.734 (0.108)	
	10N	0.014			**0.044**			0.041			0.038			0.036		
SLA (cm² g⁻¹)	0N	86.200	0.528 (0.473)		64.690	0.036 (0.850)		29.620	1.927 (0.176)		**48.340**	**11.16 (0.003)**	↑	45.880	0.85 (0.363)	
	10N	82.430			65.950			32.290			**56.050**			43.450		
Leaf Nitrogen Content (%)	0N	**2.246**	**48.65 (<0.001)**	↑	**2.738**	**8.878 (0.005)**	↑	1.329	3.332 (0.078)		1.206	1.832 (0.185)		**1.001**	**11.54 (0.002)**	↑
	10N	**3.912**			**4.026**			1.684			1.326			**1.244**		
Leaf Carbon Content (%)	0N	**42.210**	**9.438 (0.004)**	↑	38.470	2.211 (0.147)		**46.540**	**6.836 (0.014)**	↑	44.600	0.390 (0.537)		43.120	2.096 (0.157)	
	10N	**43.510**			39.260			**47.470**			44.750			43.580		
Fully Emerged Leaves	0N	**5.187**	**7.808 (0.009)**	↑	7.467	0.972 (0.332)		16.570	0.607 (0.442)		5.312	0.016 (0.901)		3.750	0.192 (0.664)	
	10N	**8.500**			8.722			14.650			5.167			4.056		
Emerging Leaves	0N	**1.250**	**11.93 (0.002)**	↑	**4.667**	**6.087 (0.012)**	↑	2.857	0.148 (0.703)		1.625	0.303 (0.586)		1.125	0.599 (0.445)	
	10N	**3.167**			**10.89**			3.353			2.111			1.444		
Senescing Leaves	0N	**1.062**	**5.332 (0.028)**	↑	2.533	1.792 (0.190)		3.714	1.195 (0.283)		6.062	1.414 (0.243)		3.875	0.037 (0.848)	
	10N	**1.889**			3.500			4.824			7.556			4.000		
Aboveground Biomass (g)	0N	**0.157**	**12.98 (<0.001)**	↑	0.3087	2.000 (0.168)		2.229	0.600 (0.445)		0.6756	0.678 (0.416)		0.7269	0.28 (0.601)	
	10N	**0.425**			0.4482			1.750			0.8294			0.8667		
Plant Height (cm)	0N	20.83	3.513 (0.070)		25.35	1.884 (0.180)		35.09	1.432 (0.241)		54.06	0.018 (0.895)		50.64	3.798 (0.060)	
	10N	24.93			28.37			28.88			54.62			57.30		
Plant Volume (cm³)	0N	**1139**	**10.25 (0.003)**	↑	**1809**	**4.686 (0.038)**	↑	5708	0.512 (0.480)		29940	0.028 (0.868)		21827	1.468 (0.235)	
	10N	**3819**			**4503**			4640			31264			31096		

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Table 4. Means and Statistical Results from Mixed-model ANOVAs Comparing Belowground Traits in Control vs 10g m⁻² N

Trait	Treatment N m⁻²	*D. oligosanthes* Means	F-Statistic (p-value)	Relationship Direction	*A. psilostachya* Means	F-Statistic (p-value)	Relationship Direction	*S. missouriensis* Means	F-Statistic (p-value)	Relationship Direction	*A. gerardii* Means	F-Statistic (p-value)	Relationship Direction	*S. nutans* Means	F-Statistic (p-value)	Relationship Direction
Focal Root Biomass (g)	0g	0.202	2.981 (0.093)		0.067	0.966 (0.333)		0.378	0.006 (0.937)		0.566	0.583 (0.450)		0.524	0.341 (0.563)	
	10g	0.314			0.084			0.367			0.691			0.471		
Total Belowground Biomass (g)	0g	2.854	1.218 (0.277)		2.221	0.370 (0.547)		3.034	1.787 (0.192)		3.265	0.428 (0.518)		3.308	1.280 (0.266)	
	10g	2.409			2.008			2.309			2.962			2.831		
SRL (m g⁻¹)	0g	7.466	0.689 (0.412)		5.765	1.426 (0.241)		4.065	0.203 (0.656)		5.142	0.843 (0.365)		7.205	0.280 (0.600)	
	10g	9.227			7.282			3.516			4.670			6.533		
Maximum Root Diameter (cm)	0g	0.068	1.981 (0.169)		0.159	0.00 (0.993)		0.336	0.024 (0.878)		0.371	1.970 (0.170)		0.446	1.970 (0.170)	
	10g	0.080			0.159			0.343			0.446			0.440		
Mean Root Diameter (cm)	0g	0.036	0.021 (0.886)		0.039	1.132 (0.295)		0.048	0.154 (0.698)		0.037	4.155 (0.050)		0.043	0.502 (0.483)	
	10g	0.036			0.032			0.046			0.046			0.048		
Root Nitrogen Content (%)	0g	**0.473**	**13.40 (<0.001)**	↑	**0.808**	**38.56 (<0.001)**	↑	**0.969**	**6.930 (0.014)**	↑	**0.557**	**25.49 (<0.001)**	↑	**0.544**	**39.72 (<0.001)**	↑
	10g	**0.741**			**1.419**			**1.407**			**1.006**			**0.992**		
Root Carbon Content (%)	0g	44.800	0.049 (0.826)		44.360	2.130 (0.154)		44.940	0.020 (0.889)		40.480	1.773 (0.192)		43.200	0.019 (0.891)	
	10g	44.580			45.070			44.840			39.140			43.290		

For N gradient dataset (**Tables 5-6**), there were similar responses to the N addition dataset. For the N gradient dataset (**Table 5-6**), each aboveground trait was significantly impacted by N addition in at least one species except for SLA which was not significantly affected by N addition for any species (**Table 5**). %N in the leaf was significant in 5 out of 5 species making it the most responsive trait. *A. psilostachya* was the most responsive to the N gradient (aboveground: 9 out of 11 = 81.8%), but in this analysis, *A. gerardii* was also largely responsive to increases in N additions (aboveground 7 out of 11 = 63.3%) (**Table 5**). In addition to significance of response, magnitude of response varied across species with *A. psilostachya* typically showing the greatest magnitude of response when a trait was significant. As with the N addition dataset, belowground traits were not often significant (**Table 6**). Most belowground traits (5 out of the 7 belowground traits: SRL, total root biomass, maximum root diameter, mean root diameter and root %C) were not significant for any species. This contrasts strongly with only a single aboveground trait (SLA) out of 11 studied, showing no significant relationship with N for all of the 5 species studied. Root %N increased with the N gradient in four of five species, with the fifth species (*S. missouriensis*) being marginally significant (**Table 6**). All significant aboveground and belowground trait responses for all species were all increases in response to N addition (**Table 5-6**).

Table 5. Means and Statistical Results of Linear Regressions on Aboveground Traits across a Gradient of N Addition (0g, 2.5g, 10g, and 20g per m^2) for 5 Species

Trait	Treatment N m^2	D. oligosanthes Means	Slope	F-Statistic (p-value)	R²	A. psilostachya Means	Slope	F-Statistic (p-value)	R²	S. missouriensis Means	Slope	F-Statistic (p-value)	R²	A. gerardii Means	Slope	F-Statistic (p-value)	R²	S. nutans Means	Slope	F-Statistic (p-value)	R²
Leaf Number	0g	10.626	0.118	3.373 (0.078)	0.094	**10.770**	**1.443**	**29.57 (<0.001)**	**0.554**	26.010	0.570	1.363 (0.256)	0.016	**10.720**	**0.434**	**7.832 (0.010)**	**0.229**	8.826	0.078	0.631 (0.436)	-0.016
	2.5g	10.920				**14.380**				27.440				**11.806**				9.020			
	10g	11.804				**25.200**				31.710				**15.065**				9.604			
	20g	12.983				**39.640**				37.410				**19.409**				10.383			
Leaf Thickness (cm)	0g	0.018	0.001	0.210 (0.651)	-0.036	**0.036**	**0.001**	**6.547 (0.018)**	**0.194**	0.045	0.001	2.336 (0.141)	0.057	0.041	0.001	0.190 (0.667)	-0.037	0.029	0.001	0.815 (0.376)	-0.008
	2.5g	0.018				**0.039**				0.046				0.041				0.030			
	10g	0.019				**0.048**				0.048				0.042				0.031			
	20g	0.020				**0.060**				0.052				0.043				0.032			
SLA (cm² g⁻¹)	0g	74.540	0.318	1.382 (0.252)	0.016	62.260	-0.521	1.601 (0.219)	0.025	27.821	0.179	2.297 (0.145)	0.056	50.520	0.237	2.175 (0.154)	0.049	45.530	-0.071	0.12 (0.733)	-0.040
	2.5g	75.330				60.960				28.269				51.110				45.350			
	10g	77.720				57.050				29.612				52.890				44.820			
	20g	80.900				51.830				31.403				55.250				44.110			
Leaf Nitrogen Content (%)	0g	**1.997**	**0.090**	**74.49 (<0.001)**	**0.762**	**2.556**	**0.077**	**16.48 (<0.001)**	**0.402**	**1.306**	**0.031**	**7.076 (0.015)**	**0.216**	**1.176**	**0.025**	**9.762 (0.003)**	**0.485**	**0.966**	**0.031**	**47.13 (<0.001)**	**0.762**
	2.5g	**2.222**				**2.748**				**1.385**				**1.217**				**1.035**			
	10g	**2.895**				**3.322**				**1.620**				**1.341**				**1.242**			
	20g	**3.793**				**4.089**				**1.933**				**1.507**				**1.518**			
Leaf Carbon Content (%)	0g	42.440	0.073	3.692 (0.068)	0.105	**37.620**	**0.087**	**7.227 (0.013)**	**0.213**	**46.050**	**0.075**	**12.91 (0.002)**	**0.351**	**44.280**	**0.046**	**5.549 (0.028)**	**0.165**	42.800	0.025	1.066 (0.313)	0.003
	2.5g	42.620				**37.840**				**46.230**				**44.400**				42.860			
	10g	43.160				**38.490**				**46.800**				**44.750**				43.050			
	20g	43.890				**39.360**				**47.550**				**45.210**				43.300			
Fully Emerged Leaves	0g	6.403	0.058	1.229 (0.280)	0.010	**6.184**	**0.470**	**15.83 (<0.001)**	**0.392**	19.411	0.018	0.018 (0.894)	-0.047	**4.815**	**0.151**	**4.928 (0.037)**	**0.146**	3.738	0.037	0.879 (0.359)	-0.005
	2.5g	6.548				**7.358**				19.457				**5.192**				3.831			
	10g	6.984				**10.881**				19.595				**6.325**				4.112			
	20g	7.565				**15.577**				19.780				**7.834**				4.486			
Emerging Leaves	0g	**2.348**	**0.044**	**4.448 (0.045)**	**0.130**	2.523	0.900	**26.33 (<0.001)**	**0.524**	0.192	0.527	1.822 (0.191)	0.036	**0.890**	**0.039**	**8.544 (0.008)**	**0.247**	1.070	0.058	1.889 (0.183)	0.037
	2.5g	**2.459**				4.772				1.125				**0.988**				0.215			
	10g	**2.791**				11.520				5.076				**1.282**				1.651			
	20g	**3.234**				20.518				10.345				**1.673**				2.231			
Scenesing Leaves	0g	1.968	0.019	0.4265 (0.521)	-0.026	2.139	0.070	1.921 (0.1797)	0.038	5.545	0.091	1.028 (0.322)	0.001	**4.990**	**0.252**	**7.95 (0.001)**	**0.232**	4.018	-0.018	0.177 (0.678)	-0.037
	2.5g	2.016				2.314				5.772				**5.622**				3.974			
	10g	2.161				2.840				6.452				**7.515**				3.842			
	20g	2.355				3.541				7.360				**10.040**				3.666			
Aboveground Biomass (g)	0g	0.371	-0.001	0.036 (0.852)	-0.044	**0.284**	**0.035**	**5.078 (0.034)**	**0.151**	3.340	0.086	1.996 (0.172)	0.043	**0.452**	**0.041**	**5.736 (0.026)**	**0.171**	**0.283**	**0.045**	**6.054 (0.022)**	**0.180**
	2.5g	0.369				**0.370**				3.553				**0.554**				**0.395**			
	10g	0.362				**0.630**				4.195				**0.858**				**0.732**			
	20g	0.352				**0.977**				5.050				**1.265**				**1.180**			
Plant Height (cm)	0g	27.006	-0.063	0.15 (0.702)	-0.038	**23.040**	**0.417**	**13.27 (0.001)**	**0.348**	47.650	-0.086	0.093 (0.764)	-0.043	53.130	0.367	1.723 (0.203)	0.030	**50.730**	**0.758**	**4.974 (0.036)**	**0.147**
	2.5g	26.849				**24.083**				47.440				54.050				**52.630**			
	10g	26.378				**27.211**				46.790				56.800				**58.310**			
	20g	25.751				**31.382**				45.930				60.470				**65.900**			
Plant Volume (cm³)	0g	2004.2	36.850	0.431 (0.518)	-0.025	**882.0**	**249.1**	**28.25 (<0.001)**	**0.542**	7546.0	132.3	1.013 (0.326)	0.001	25955.0	480.9	0.638 (0.433)	-0.016	23077.0	2186.0	1.788 (0.195)	0.033
	2.5g	2096.3				**1504.0**				7876.0				27157.0				28542.0			
	10g	2372.7				**3373.0**				8868.0				30763.0				44936.0			
	20g	2741.1				**5864.0**				10191.0				35572.0				66795.0			

Note. Bold values indicate statistical significance according to an alpha value of 0.05, adjusted for multiple comparisons.

Table 6. Means and Statistical Results of Linear Regressions on Belowground Traits across a Gradient of N Addition (0g, 2.5g, 10g, and 20g per m²) for 5 Species

Trait	Treatment N m⁻²	D. oligosanthes Means	Slope	F-Statistic (p-value)	R²	A. psilostachya Means	Slope	F-Statistic (p-value)	R²	S. missouriensis Means	Slope	F-Statistic (p-value)	R²	A. gerardii Means	Slope	F-Statistic (p-value)	R²	S. nutans Means	Slope	F-Statistic (p-value)	R²
Focal Root Biomass (g)	0g	0.178	0.002	0.140 (0.715)	-0.039	**0.038**	**0.005**	**9.542 (0.006)**	**0.280**	0.520	0.015	1.812 (0.193)	0.036	0.591	-0.003	0.055 (0.818)	-0.045	0.521	0.001	0.039 (0.845)	-0.044
	2.5g	0.182				**0.050**				0.558				0.585				0.524			
	10g	0.195				**0.086**				0.673				0.566				0.532			
	20g	0.211				**0.134**				0.826				0.541				0.544			
Total Belowground Biomass (g)	0g	3.521	-0.059	1.584 (0.221)	0.025	2.645	0.005	0.023 (0.880)	-0.046	3.803	-0.027	0.343 (0.564)	0.031	3.435	-0.002	0.002 (0.969)	-0.048	4.190	-0.051	1.529 (0.229)	0.022
	2.5g	3.374				2.658				3.736				3.430				4.062			
	10g	2.930				2.695				3.536				3.418				3.677			
	20g	2.338				2.744				3.269				3.402				3.164			
SRL (cm² g⁻¹)	0g	9.659	0.008	0.002 (0.963)	-0.045	6.124	-0.040	0.100 (0.760)	-0.043	1.906	0.002	0.009 (0.925)	-0.047	4.503	-0.023	0.184 (0.673)	-0.039	7.899	-0.111	0.994 (0.329)	-0.001
	2.5g	9.679				6.024				1.911				4.447				7.622			
	10g	9.738				5.723				1.926				4.278				6.789			
	20g	9.816				5.322				1.947				4.052				5.678			
Root Nitrogen Content (%)	0g	**0.459**	**0.035**	**28.49 (<0.001)**	**0.545**	**1.018**	**0.028**	**7.969 (0.010)**	**0.241**	1.048	0.023	3.707 (0.068)	0.110	**0.554**	**0.035**	**44.12 (<0.001)**	**0.652**	**0.489**	**0.046**	**92.33 (<0.001)**	**0.799**
	2.5g	**0.547**				**1.088**				1.105				**0.642**				**0.604**			
	10g	**0.812**				**1.297**				1.274				**0.906**				**0.950**			
	20g	**1.166**				**1.576**				1.499				**1.258**				**1.411**			
Root Carbon Content (%)	0g	45.010	-0.017	0.037 (0.849)	-0.044	44.450	0.068	2.481 (0.130)	0.063	44.560	-0.045	1.857 (0.187)	0.037	39.660	0.057	0.413 (0.527)	-0.026	41.900	0.018	0.094 (0.762)	-0.041
	2.5g	44.960				44.620				44.450				39.800				41.950			
	10g	44.840				45.130				44.120				40.220				42.080			
	20g	44.670				45.810				43.670				40.790				42.260			
Maximum Root Diameter (cm)	0g	0.093	0.001	0.263 (0.613)	-0.033	0.126	0.003	4.115 (0.055)	0.124	0.126	0.002	0.261 (0.615)	-0.035	0.379	0.003	0.919 (0.349)	-0.004	0.379	0.004	1.646 (0.213)	0.027
	2.5g	0.095				0.134				0.134				0.387				0.387			
	10g	0.100				0.159				0.159				0.411				0.411			
	20g	0.107				0.192				0.192				0.443				0.443			
Mean Root Diameter (cm)	0g	0.029	0.001	1.942 (0.177)	0.039	0.037	0.001	0.141 (0.711)	-0.041	0.052	0.001	0.002 (0.962)	-0.048	0.045	0.001	0.460 (0.505)	-0.026	0.043	0.001	0.175 (0.680)	-0.037
	2.5g	0.030				0.038				0.052				0.046				0.043			
	10g	0.031				0.039				0.052				0.048				0.043			
	20g	0.034				0.040				0.052				0.051				0.044			

Note. Bold values indicate a statistically significant p-value according to an alpha value of 0.05, adjusted for multiple comparisons.

Overall, aboveground traits were overwhelmingly more responsive than belowground traits with N additions (**Table 3-6**; **Fig. 8**), particularly so with the N gradient analysis. Additionally, the different species showed different aggregates of responsiveness with the two forb species being at opposite extremes. *S. missouriensis* was the least responsive to N additions and its cumulative effects. Conversely, *A. psilostachya* was most responsive, with the greatest number of aboveground and belowground traits showing significant change. Of the grasses studied, the most responsive species varied between the two datasets with *A. gerardii* being the most significant aboveground response across N gradients, and *D. oligosanthes* being the most responsive in the N addition dataset.

Figure 8. Percentage of Traits Responsive to N in Statistical Tests for All Focal Species
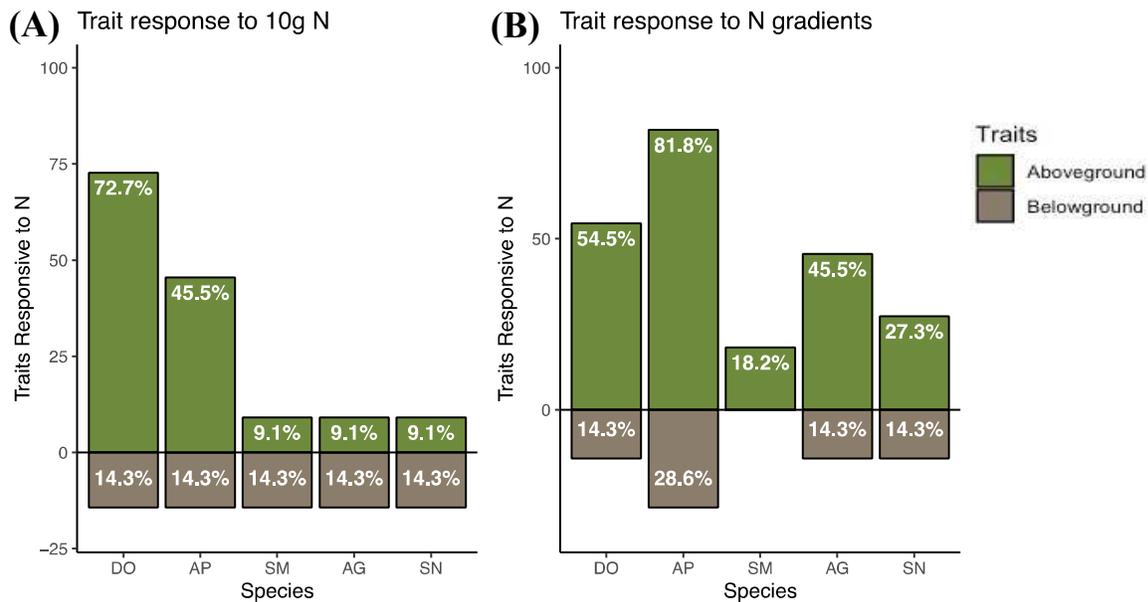
Note. Percentage of traits (aboveground=green, belowground=brown) responsive to N in **(A)** the N addition dataset (mixed model ANOVAs of 0g vs 10g N m^{-2}) and **(B)** the N gradient dataset (linear regressions across N gradients of 0g, 2.5g, 10g, and 20g m^{-2}) for all focal species. Species respond differently to the amount of N added. Overall, plant traits are more responsive to N additions aboveground as compared to belowground.

3.2 Q2: Trait Space Response to Nitrogen

Trait space was mostly unresponsive to N addition and to the N gradient (**Fig. 9**, **Table 7**). Only *A. gerardii* showed significant trait space responses to N addition (**Fig. 9D**, left) and the N gradient (**Fig. 9D**, right) with only the 10g N m^{-2} treatment being significantly different from the control. Similarly, *A. gerardii* also was one of only two species (other was *D. oligosanthes)* to show significant differences in dispersion for the N addition analysis (**Table 8-9**). All the remaining species failed to refute the null hypothesis of homogeneity of variance in multivariate space (betadisper test).

Table 7. Explanation of Trait Variation Contributed by Axes in Principle Component Analysis (PCA) and Permutational Multivariate Analysis of Variance based on Distance (PERMANOVA) in Testing the Effect of N Addition and N Gradients for 5 Species

| Species | N Addition | | | N Gradients | | |
| | Variation (%) Explained by | | PERMANOVA | Variation (%) Explained by | | PERMANOVA |
	PC 1	PC 2	R² (p-value)	PC 1	PC 2	R² (p-value)
D. oligosanthes	27.5%	21.0%	0.680 (0.760)	32.6%	22.5%	0.180 (0.192)
A. psilostachya	29.2%	23.8%	0.034 (0.304)	23.1%	22.5%	0.224 (0.121)
S. missouriensis	25.5%	17.9%	0.058 (0.137)	24.6%	21.2%	0.101 (0.742)
A. gerardii	**31.5%**	**19.5%**	**0.179 (<0.001)**	**36.6%**	**24.2%**	**0.224 (0.043)**
S. nutans	28.4%	19.1%	0.034 (0.31)	30.6%	17.1%	0.159 (0.258)

Note. Bold values indicate a statistically significant p-value according to an alpha value of 0.05, adjusted for multiple comparisons.

Figure 9. Response of Plant Traits to N Addition using Principal Component Analysis (PCA) of Plant Traits based on Bray-Curtis Distance Comparing 0g vs 10g N m^{-2} (left) and Comparing N Gradients of 0g, 2.5g, 10g, and 20g per m^2 (right)
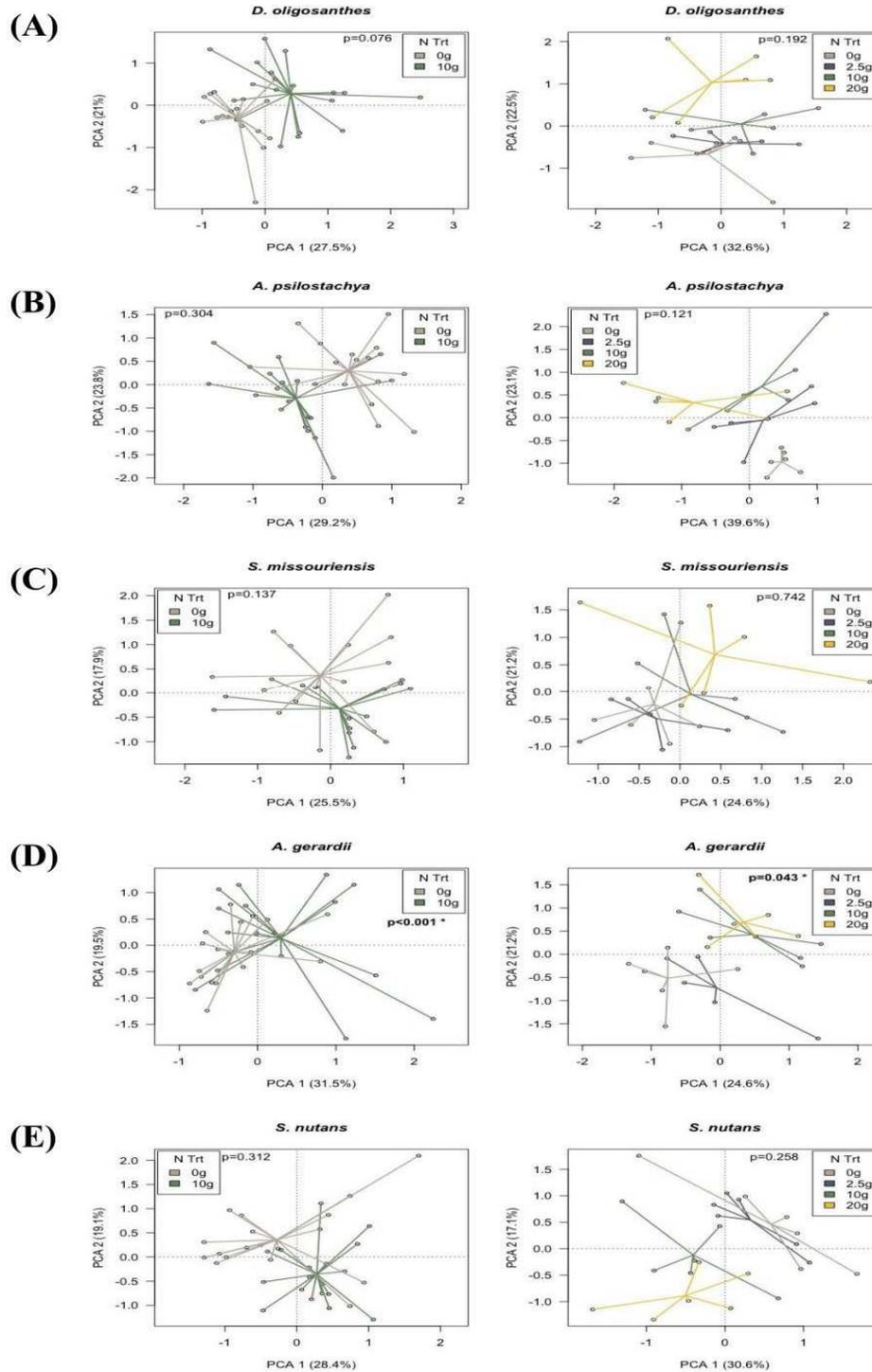
Note. Coloring is set by treatment. Each panel is a different species, **(A)** *D. oligosanthes*

(B) *A. psilostachya* **(C)** *S. missouriensis* **(D)** *A. gerardii* and **(E)** *S. nutans*. According to a

PERMANOVA test, the only species with a significant change in trait space was *A. gerardii*.

Functional dispersion which is a metric of functional diversity that explores how the distribution of traits in trait space maximizes divergence in trait characteristics (Mason et al. 2005) increased in *D. oligosanthes* (60.9%), *S. missouriensis* (18.8%), and *A. gerardii* (305.8%) and decreased in *A. psilostachya* (15.1%) and *S. nutans* (22.0%) when analyzing the N addition dataset (**Table 8**). Functional richness (FRic) which measures the amount of niche space filled by a species in the community (Mason et al. 2005) increased in *D. oligosanthes* (3,448.3%), *A. psilostachya* (273.9%), and *A. gerardii* (338.2%) while *S. missouriensis* (68.8%) and *S. nutans* (29.5%) showed a decrease (**Table 8**). Functional dispersion across the N gradient was not linear (**Table 9**). Functional dispersion increased in *D. oligosanthes* for each N treatment compared to control with the greatest increase occurring in the 10g N m^{-2} (**Table 9**). Functional diversity also increased in *A. gerardii* for each treatment relative to the control but the greatest increased occurred in the 2.5 g N m^{-2}. Functional diversity for both *S. missouriensis* and *S. nutans* decreased with each treatment relative to the control with the greatest decrease occurring in the 10g N m^{-2}. Functional diversity for *A. psilostachya* increased in the 2.5g N m^{-2} and 10g N m^{-2} treatments but decreased in 20g N m^{-2} compared to the control.

Table 8. Effects of N addition (0 vs 10g N m^{-2}) on Functional Dispersion (FDis), Functional Richness (FRic), and Beta-diversity (Betadisper) of Traits for 5 Species

Treatment N m^{-2}	*D. oligosanthes*			*A. psilostachya*			*S. missouriensis*			*A. gerardii*			*S. nutans*		
			Betadisper			Betadisper			Betadisper			Betadisper			Betadisper
	FDis	FRic	F (p-value)	FDis	FRic	F (p-value)	FDis	FRic	F (p-value)	FDis	FRic	F (p-value)	FDis	FRic	F (p-value)
0g	9.57	0.12	**4.529 (0.037)**	18.14	11.10	0.189 (0.667)	6.54	25.60	0.953 (0.335)	5.45	0.75	**5.155 (0.021)**	7.77	3.12	0.413 (0.565)
10g	15.40	41.5		15.96	41.50		7.77	7.99		22.13	3.33		6.06	2.20	

Note. Bold values indicate a statistically significant p-value according to an alpha value of 0.05, adjusted for multiple comparisons.

Table 9. Effects of N Addition across a Gradient (0g, 2.5g, 10g, and 20g per m^2) on Functional Dispersion (FDis), Functional Richness (FRic), and Beta-diversity (Betadisper) of Traits for 5 Species

Treatment N m^{-2}	D. oligosanthes		A. psilostachya		S. missouriensis		A. gerardii		S. nutans	
	FDis	Betadisper F (p-value)	FDis	Betadisper F (p-value)	FDis	Betadisper F (p-value)	FDis	Betadisper F (p-value)	FDis	Betadisper F (p-value)
0g	6.49	0.093 (0.969)	6.98	0.887 (0.465)	5.02	0.621 (0.613)	3.30	0.080 (0.965)	10.38	0.540 (0.660)
2.5g	8.88		11.76		4.44		7.07		5.45	
10g	12.41		21.28		3.74		3.88		4.58	
20g	6.99		6.56		4.27		5.86		6.32	

Note. Bold values indicate a statistically significant p-value according to an alpha value of 0.05, adjusted for multiple comparisons.

3.3 Q3: Above- vs Belowground Trait Relationships Across Species

In general, there was a lack of statistically significant trade-off in above and belowground traits with the majority of significant correlations being positive (**Appx. A1-5**). *Sorghastrum nutans* (**Appx. A5**) had more correlated above-belowground pairs overall than any other species and also showed the highest rate of negative correlations or tradeoffs. Additionally, high N addition treatments (10g and 20g N m^{-2}) had more pairs of above- and belowground traits that were significantly related in all species when compared to the control and 2.5 g N m^{-2} treatments. High N treatment of 20g N m^{-2} showed the strongest correlations with all significant aboveground-belowground trait pairs reporting very high correlations (r > 0.8) (**Appx. A1-5**, pink shaded plots). Below I select three pairs of above and belowground traits (**Appx. A1-5**, pink shaded plots with orange box) where tradeoffs would be hypothesized for further exploration: (1) aboveground biomass vs belowground biomass, (2) SLA vs SRL, and (3) leaf %N vs root %N.

Trait Pair 1: Aboveground biomass vs belowground biomass - All species had an overall significant positive correlation between aboveground biomass and root biomass with N additions (**Appx. A1-5**, pink plots with orange box). Increased levels of N caused this trait relationship to strengthen for some species (*D. oligosanthes*, *A. psilostachya* and *S. missouriensis*) but weaken for others (*A. gerardii* and *S. nutans*). Across species, no trade-offs were found between above and belowground biomass.

Trait Pair 2: Specific Leaf Area (SLA) vs Specific Root Length (SRL) - Only *A. psilostachya* had a significant correlation between SLA and SRL (**Appx. A1-5**, pink plots with orange box). The strength of the relationship was significant, although weak across all treatments (Pearson's: $r = 0.336$, $p < 0.05$). With increasing N additions, the correlation between SLA and SRL for *A. psilostachya* strengthened moderately, although the relationship was still weak (but significant, $p < 0.05$). Across species, no trade-offs were found between SLA and SRL.

Trait Pair 3: Leaf N vs root N - An overall positive correlation between leaf %N vs root %N was found in all species (**Appx. A1-5**, pink plots with orange box). A weaker overall relationship was found in *A. gerardii* and *S. missouriensis*, but both species showed an increase in correlation strength with increasing N additions. *S. nutans* showed the strongest correlation between leaf %N vs root %N with an overall Pearson's $r = 0.880$ ($p < 0.001$). No trade-offs were found between leaf %N vs root %N, but strong, increasing correlations with N additions reflect aboveground-belowground resemblance in N tissue levels.

CHAPTER IV: DISCUSSION

4.1 Q1: How do plant traits - in particular, root traits - change with N availability?

Overall, belowground traits showed little change with N additions in comparison to aboveground in our analyses of 5 common grassland species. Dominant species of grassland communities are commonly high-acquisition on the Resource Economics Spectrum (Prieto et al. 2015), using nutrient uptake strategies that facilitate rapid regrowth and high turnover rate with high SLA (Lavorel and Garnier 2002, Li et al. 2017) and high root %N (Roumet et al. 2006). Grasses and forbs generally use a "fast" return on investment strategy (Valverde-Barrantes et al. 2020) which might reduce the potential for root trait variation. With N additions, the minimal to no difference in growth-related belowground traits (SRL, root mean diameter, maximum root diameter, focal root biomass) across species suggests that global change factors like N deposition do not constrain plant nutrient uptake strategy to a large extent. These results are consistent with other intraspecific trait studies in N additions which found moderate to high dissimilarity in aboveground traits and little to no dissimilarity in belowground traits where N addition had little effects on root production (Carmona et al. 2021, Moreau et al. 2017, Yan et al. 2021).

Although I did see trait responses, there were not as many as I had initially expected and there were few to none belowground. Long-term additions may explain why we do not see as many responses. Effects of long-term (≥ 10 yr) nutrient addition are shown to differ from short-term (< 10 yr) nutrient additions effects on traits and plant community (La Pierre and Smith 2015, Komatsu et al. 2019). Short term N effects on ecosystem function tend to be through changes in the individual plant, where long term effects tend to be through changes in the plant community composition or the population structure (Koerner et al. 2016, Avolio et al. 2014).

Additionally, a link has been made between morphological plasticity and plant "memory" of long-term processes (Ren et al. 2017). Plants with chronic or long-term nutrient additions do not respond as dramatically in comparison to short-term, where the (long-term addition) plant has "memory" of resource stability and is able to adjust efficiency of nutrient allocation (Jing et al. 2021). The lack of response by belowground traits may be due to long-term effects of global change factors (Read et al. 2017). Long-term effects of nutrient additions on traits are not fully understood (Seabloom et al. 2020) but have been identified as playing a role in shifting trait dynamics as compared to short-term (La Pierre and Smith 2015). The results of my study show low root growth response in N additions with no change in mean root diameter and maximum root diameter, and mostly no change in belowground focal root biomass where grassland roots have a high turnover rate where it is not necessary to invest in short-lived root structures (Gill and Jackson 2000). Consistent nutrient availability in the long-term experiments (P-Plots, Nutnet, and ChANGE) may contribute to lack of belowground changes where root plasticity is shown to change with heterogeneous nutrient availability (Hodge 2004).

With N additions, all species resulted in significant increases in root %N, this is consistent with plants with high nutrient acquisition strategies taking in additional nitrogen when available as long as sufficient water is also available (Plett et al. 2020). Across species, total root biomass did not change with N additions in my study. Belowground trait space has been demonstrated to be shared across species with differentiation of trait space aboveground with N additions (Carmona et al. 2021). Carbon content in root tissue did not change with N for all species. N additions are shown to increase root turnover rate thereby directly increasing root C input into the soil (Yan et al. 2021, Wang et al. 2018) but not necessarily standing root biomass at any one timepoint.

The only species to show a morphological (size-related) change in any root trait was *Ambrosia psilostachya* with an increase in focal root biomass in response to N. There is a lack of belowground and/or trait studies on *A. psilostachya* (Western Ragweed), a common native forb of the tallgrass prairie (Gillen and McNew 1987, Vermeire and Gillen 2000). Other species of the genus *Ambrosia* (*e.g., A. trifida*, *A. artismilfolia)* are widely studied as invasive species outside of the United States and are shown to have variation in genetics, morphology, physiology (Sun and Roderick 2019, Hovick et al. 2018). Generally, *Ambrosia* plants are shown to have very high trait plasticity, being able to respond rapidly to environmental pressures and heterogeneity conditions (Esipenko et al. 2020, Gentil et al. 2021). Highly plastic, *A. psilostachya* showed the most variability in trait response through changes in aboveground traits and a single belowground trait in N additions.

It is well published that plants growing in nutrient-rich environments generally grow leaves with high N contents and a relatively short lifespan producing large amounts of nutrient-rich litter from senesced plant tissue (Wright et al. 2004, Westoby et al. 2002). My results are consistent with this, where across species, there was a large response to N in leaf traits, with increase in leaf %N and changes in traits related to leaf stage (number of emerging leaves, number of fully emerged leaves, number of senescing leaves) with increasing N due to a high turnover rate. Across all study species, plant height and SLA (apart from *S. nutans*) remained unchanged across treatments. Although there are a few studies that support this finding (Cui et al. 2020), it largely conflicts with other studies, including a global meta-analysis by Xingyun et al. (2020) that showed a positive response by growth-related aboveground traits to N additions. Interestingly, this meta-analysis also highlighted a trend where long-term N additions may result in diminishing response of some traits with increasing N levels and experimental duration

(Xingyun et al. 2020).

A strong linear relationship between leaf %N and N gradient was significant across all species which includes three dominant grasses: *D. oligosanthes*, *A. gerardii* and *S. nutans*. This finding conflicts with a study by Yu et al. (2015) that argues that dominant grasses are very homeostatic (*e.g.*, not responsive) in tissue %N with nitrogen additions. Perhaps my result that leaf %N increases with N additions, may be attributed to the cumulative effects of N addition. A study by Sun et al. (2022) found cumulative effects of N additions with some trait responses showing trends with results opposite to their own findings. Trait responses differed across sampling years with variation across species (Sun et al. 2022).

The lack of differences in belowground traits with N additions suggest that the current focus on aboveground trait responses might be well justified. Labor intensive and time-consuming root sampling in future grassland studies are not as critical for understanding plant response to N additions. The higher %N in root tissue suggests that there might be potentially higher root turnover, and this finding suggests that great focus on turnover of belowground biomass may be important for understanding nutrient effects on tallgrass prairie carbon sequestration.

4.2 Q2: Does nitrogen availability influence the whole-plant niche space of a species and thus its functional role in the community?

Only one species, *A. gerardii* showed a significant change in trait space as the result of N additions with no change in trait space reported for any of the other species studied. A perennial C4, clonal grass, *A. gerardii* is a dominant and abundant species across my study site at Konza Prairie Biological Station as well as across the entire Great Plains grassland ecoregion. Regionally common, dominant species are members of a plant community with high local

relative abundance (Gaston 2010) and have a large functional impact on the ecosystem (Avolio et al. 2019). The functional impact of dominant species is dependent on their specific functional traits (Avolio et al. 2019, Hillebrand et al. 2008). Multiple studies (Bachle et al. 2018, Hoffman and Smith 2020) suggest the unique trait variability of *A. gerardii* to be a mechanism that in fact, enables and propels the dominance of this species across grassland communities. Additionally, trait variability is documented to lead toward a more stable system due to niche stabilization which affects community composition and ecosystem function (Turcotte and Levine 2016).

Dominant species are commonly generalists- meaning they are able to survive across a broad range of environmental conditions. There is support that *A. gerardii* shares characteristics of species in the category of "generalists" (Bachle et al. 2019). Adaptive trait variability permits greater phenotypic plasticity, which provides population buffering for some species that exist across broad climate gradients. Trait plasticity and variability in *A. gerardii* contributes to its successful dominance by altering traits to fit new environmental conditions. Both functional diversity and functional richness of *A. gerardii* increased with 10g N m^{-2} additions, proposing promising adaptability of a dominant grassland species to future global change as levels of global N deposition steadily increase.

I explored three metrics of trait diversity: betadisper, functional divergence, and functional richness. All three essentially are different metrics to represent how much of the niche space a species fills in a community (Wilson et al. 2015). Changes in functional richness differs across species in response to N. Significant changes in individual trait response for *D. oligosanthes* and *A. gerardii* are supported by increases in the magnitude of functional niche space occupied by those individuals with N additions. Previous studies have suggested a link between positive responses in plant traits (*e.g.*, N tissue content, biomass and other growth-

related traits) and increases in functional richness with long-term N additions (Roscher et al. 2019). My results showed species-specific variability in functional diversity with N additions. A study that contradicts my findings by (Li et al. 2019) shows little to no changes in functional diversity with N additions. My findings suggest that *D. oligosanthes* and *A. gerardii* may take up a larger portion of the niche space in high nitrogen global change scenarios of the future. If so, this might increase function of things like biomass but also decrease species richness of this community.

4.3 Q3: Do belowground trait responses reflect tradeoffs between above and belowground traits?

Across species there was a variety of changes in aboveground traits with N additions, yet all tell the same story belowground with little to no response in root traits. An intraspecific trait study by Carmona et al. (2021) shows similar results where aboveground trait response does not reflect belowground trait response, regardless of category, quantity, or composition of traits changing aboveground. The aboveground and belowground planes are fundamentally decoupled in functional trait space, so that the aboveground phenotype of a species does not provide much information on its what is going on belowground in the roots (Yang et al. 2022). Carmona et al. (2021) also found that species tend to partition the aboveground trait space and share the root trait space with other species.

Correlation of traits showed intraspecific variability. For example, pairs of traits were well correlated in *S. nutans*, so we might be able to infer belowground processes from the aboveground response for *S. nutans*, but this does not translate to other species- grass or forb-regardless of functional similarity. This contrasts with other studies describing a trend of some degree of coordination between above and belowground (Reisch 2014, Shen et al. 2019).

However, limitations to assuming this link is applicable on a broader scale is heavily disputed (Valverde-Barrantes et al. 2017, Bergmann et al. 2017, Ma et al. 2018). A paper by Agrawal (2019) demonstrated the need for standardizing trait studies when studying and comparing plant trade-offs where trait selection and methodology of trait collection is highly variable across studies. A global meta-analysis by Weigelt et al. (2021) attempts to synthesize trait analyses to determine if aboveground and belowground are linked, yet this synthesis was limited in quantity of root studies conducted and in analogous root traits across studies. There are mixed results for above and belowground coordination where it sometimes depended on the trait studied and some evolutionary drivers are unique to root traits (Bergmann et al. 2020).

Trait correlation changed across the N treatments gradients. Strength of correlation increased as N level increased where traits became more correlated in 20g N m^{-2} as compared to the control. My results suggest that long-term nitrogen application leads to closer relationships between traits and is supported by previous literature (Wright and Cannon 2001, Pensa et al. 2010, Xingyun et al. 2020). The effects of long-term N addition in a leaf trait correlation study by Sun et al. (2022) were significantly higher than short-term treatments, which indicates cumulative effects of N deposition are important. In ambient conditions, aboveground does not accurately predict belowground and strength of correlations changes over time (Sun et al. 2022). Perhaps as global change processes like N deposition alter individuals, certain traits become more correlated because the pressure of the global change drivers is steering the whole-plant's traits in a single direction, overriding individual trait plasticity. The threshold response of plants (Zong et al. 2019) could contributing to why long-term nutrient effects studies are seeing traits respond differently than initially reported (Jing et al 2021).

No trade-offs were found with Pearson's correlation analysis for the three highlighted aboveground-belowground trait pairs studied. Interestingly, trait relationships strengthened with increasing N for two trait pairs, aboveground biomass vs root biomass and leaf %N vs root %N. A recent study by Chen et al. (2021) supports this finding that the strength of the relationship increased between N content in plant tissues with increasing N levels. Aboveground N content was predictive of belowground N content as this relationship (leaf %N vs root %N) was correlated across species. The aboveground biomass vs root biomass trait relationship weakened with N additions for *A. gerardii* and *S. nutans*. My results provide further support for previous studies (Craine et al. 2005) that aboveground biomass can be used as a proxy for belowground biomass in control conditions. However, N addition alters these relationships for *D. oligosanthes*, *A. psilostachya* and *S. missouriensis* by strengthening the correlation, becoming more predictive. Trait correlations and predictability for two trait pairs studied were species dependent. SLA vs SRL was mostly not correlated in control conditions and/or with N additions. There was no strong correlation found across species where aboveground was not predictive of belowground when comparing SLA vs SRL. This is surprising because many studies have confidently used SLA vs SRL to a be a complementary trait pair (Liu et al. 2010). Conversely, some studies also support this finding of belowground decoupled from aboveground (Cardou et al. 2022, Craine et al. 2005). A common theme of recent root trait studies is the that understanding of roots had been largely overestimated including in predictability of response to factors like global change (Tumber☐Dávila et al. 2022).

CHAPTER V: CONCLUSION

5.1 Conclusion

This thesis provides critical understanding of the effects of nutrient deposition on plant traits for five common and abundant species in tallgrass prairie, helping to increase the predictive power of how tallgrass prairie function may change under future anthropogenic forces through three key findings. First, this study showed that belowground traits lacked response to N additions except for %N in root tissue. This suggests that commonly measured root traits may not be helpful for understanding changes in function like carbon cycling, but that instead carbon cycling may be more influence by things like root turnover and aboveground carbon inputs. Second, individual trait responses (Q1) as well as responses of metrics of trait diversity (Q2) were species specific. *A. psilostachya* was the most responsive to N additions with the greatest magnitude of trait response, and *A. gerardii* was the most responsive in trait space increasing its niche space with N addition. These species specific responses will make generalizing responses N addition difficult and potentially make including trait responses in ecosystem models impossible. Third, above and belowground traits were positively correlated suggesting similar responses above and belowground in opposition to trade-offs as hypothesized. Additionally, the strength of these correlations increased with N addition. This suggests that aboveground traits are a good predictor of belowground trait values and that N addition may only increase that predictability. Overall, this study contributes greatly to further understanding of plant trait responses to N addition in tallgrass prairie and increases our predictive power of what tallgrass prairie will look like and how it will function in the novel environmental scenarios of the future.

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Appendix A. Pearson correlation analysis among traits of 5 species across nitrogen additions for **(1)** *D. oligosanthes* **(2)** *A. psilostachya* **(3)** *S. missouriensis* **(4)** *A. gerardii* and **(5)** *S. nutans.* Below is a correlogram or generalized pairs plot showing, below the diagonal, all the pairwise scatter plots comparing each pair of traits. Above the diagonal are correlation statistics (Pearson's product- moment correlation, r) and the significance of the least-squares regression (p-value). Different colors represent gradients of N additions per m^{-2}, 0g (control), 2.5g, 10g, and 20g. Above- and belowground trait pairs are shaded in pink and the 3 focal trait comparisons examined in this study are shown with an additional orange box.

**(1)**

Correlogram for DO with ggpairs()
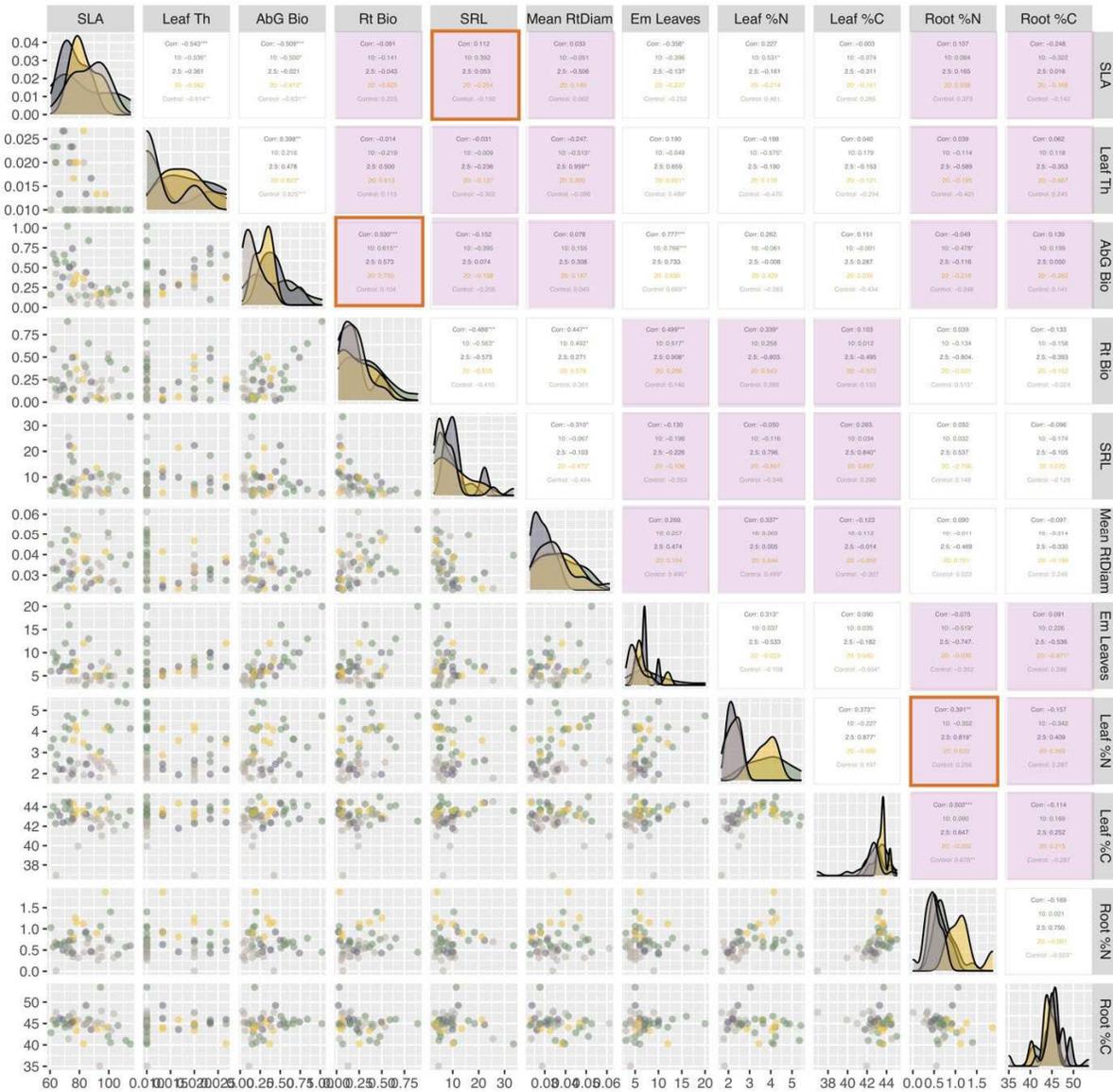
(2) Correlogram for AP with ggpairs()
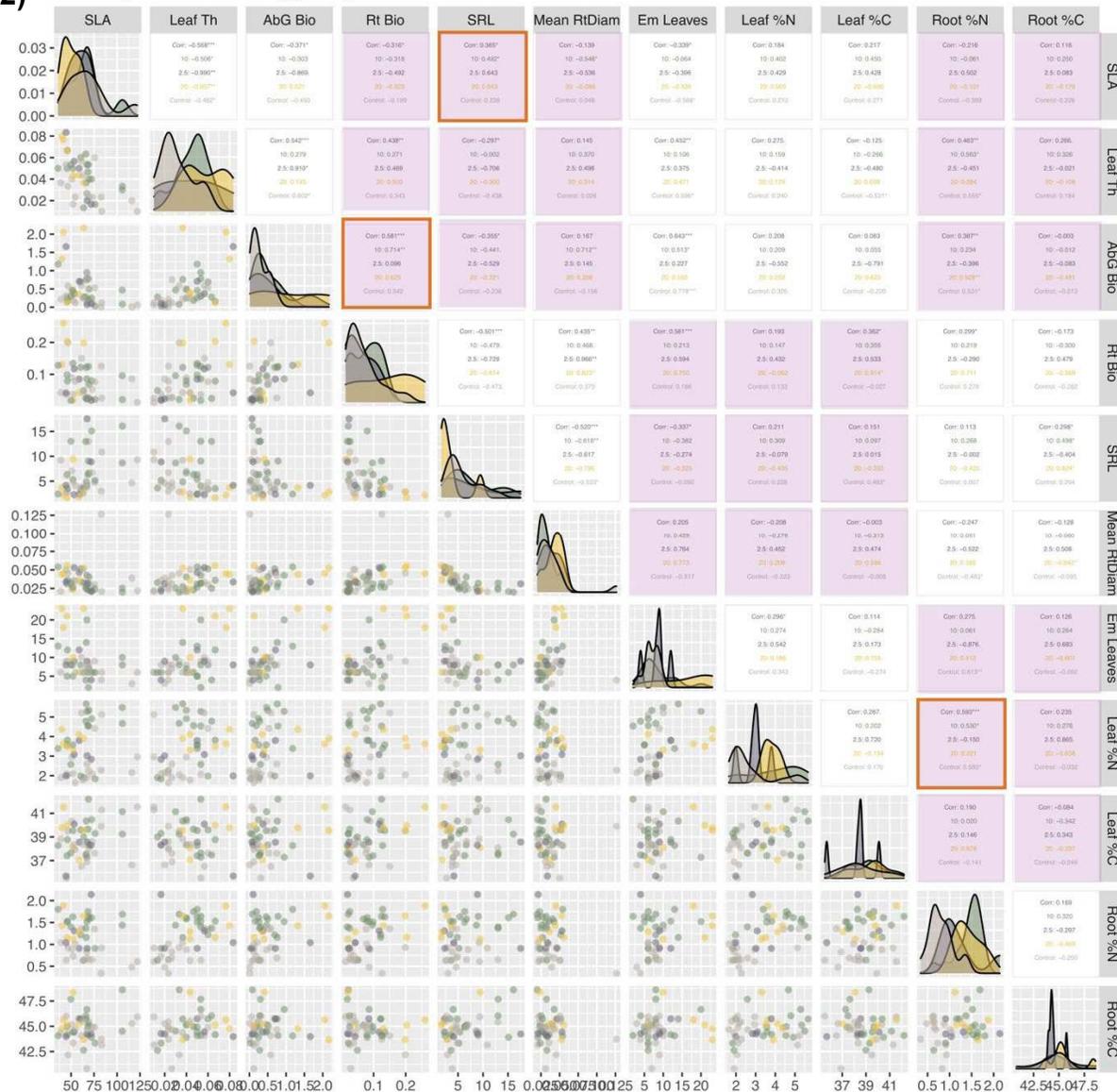
(3) Correlogram for SM with ggpairs()
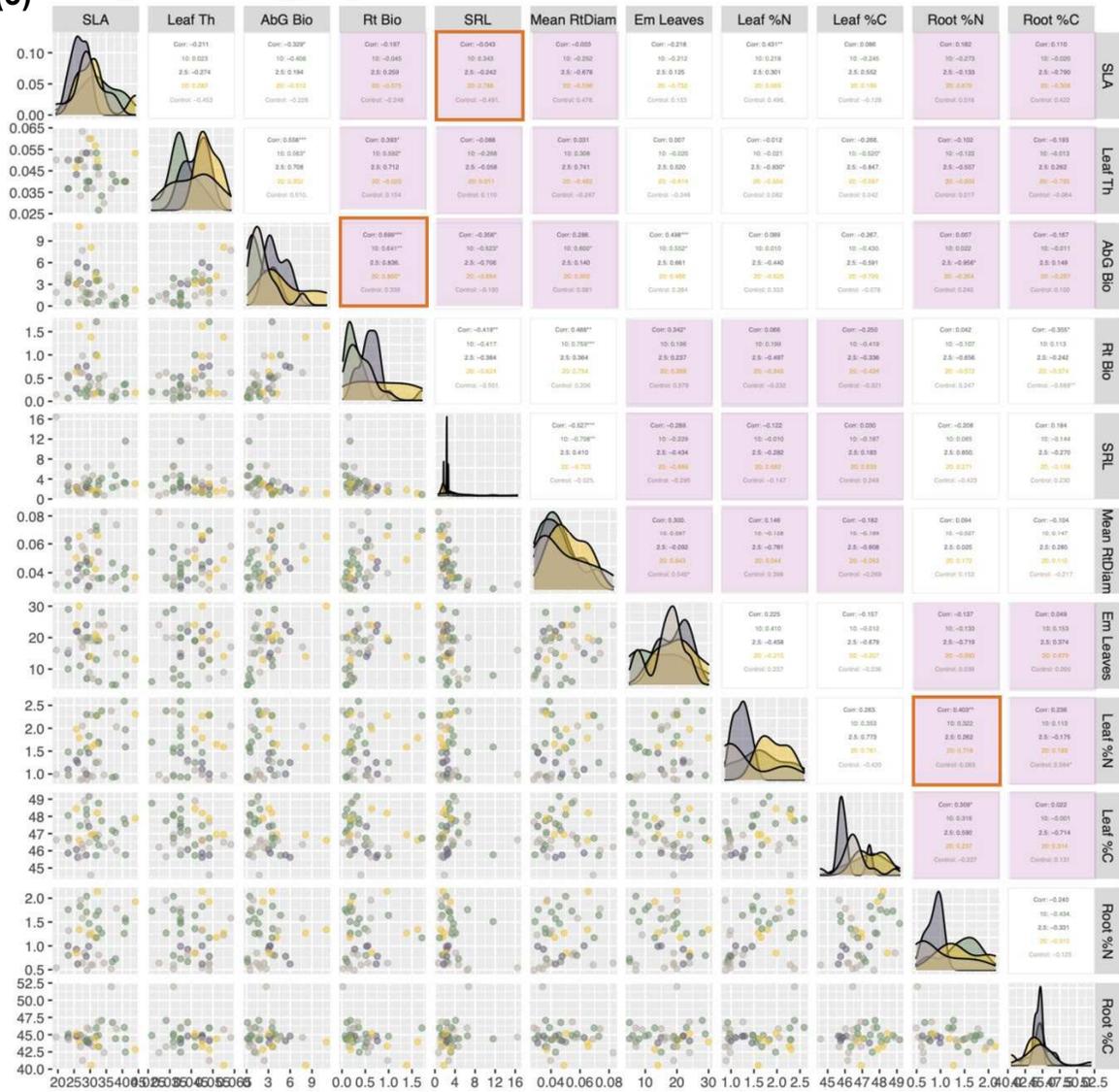
(4) Correlogram for AG with ggpairs()
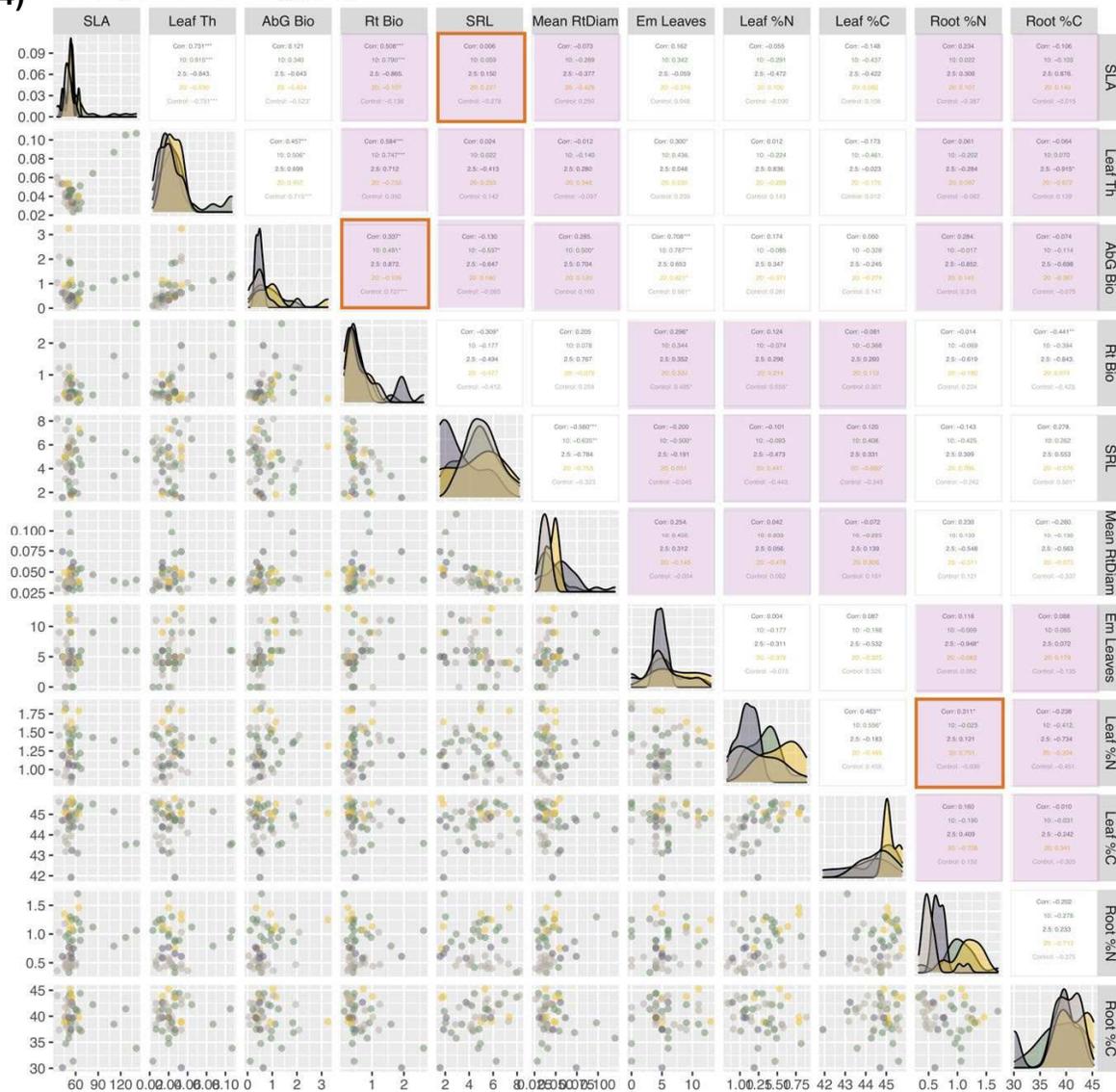
(5) Correlogram for SN with ggpairs()
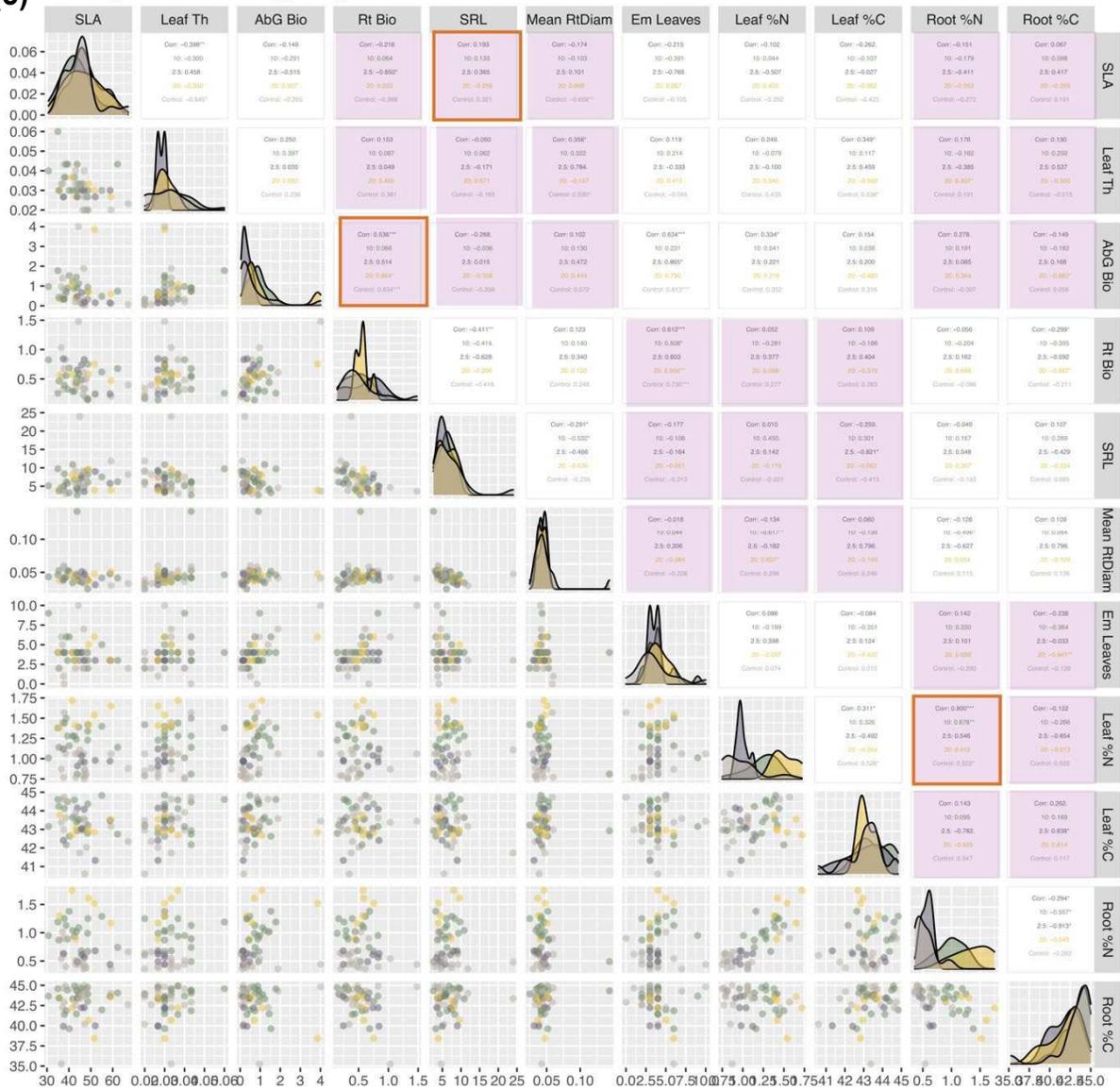
APPENDIX B: TRAIT DEFINITIONS AND FORMULAS

- **<u>Plant Volume:</u>** Spatial area that a plant takes up aboveground

$$Vol\ cm^3 = Height\ (cm) * Width\ 1\ (cm) * Width\ 2\ (cm)$$

- **<u>Specific Leaf Area (SLA):</u>** Leaf area to mass ratio

$$SLA\ cm^2 g^{-1} = \frac{(Leaf\ Area\ (cm^2))}{(Leaf\ Dry\ Weight\ (g))}$$

- **<u>Specific Root Length (SRL):</u>** (Fine) root length to mass ratio

$$SRL\ m\ g^{-1} = \frac{(Root\ Length\ (m))}{(Root\ Dry\ Weight\ (g))}$$

- **<u>Total Root Biomass:</u>** Total core biomass

$$Total\ Root\ Biomass\ (g) = Focal\ Root\ Biomass\ (g) + Net\ Root\ Biomass\ (g)\ in\ Soil\ Core\ Sample$$

- **Functional Dispersion (FDis):** measure of a species' niche breadth

  $fdisp(d, a, tol = 1e - 07)^*$

  > **d** = an individual-by-individual distance matrix computed from traits. NAs not allowed.
  >
  > **a** = matrix containing the abundances of the individuals of a species in d (or presence, e.g., 1). Rows are N treatments and individuals are columns. The number of individuals of a species (columns) in a must match the number of individuals of a species in d.
  >
  > **tol** = tolerance threshold to test whether the distance matrix is Euclidean

  *Code modified from de Bello (2011) paper using fdisp function in the FD (version 12.1) package.

- **Functional Richness (FRic):** measure of a species' trait niche space in a community

  $fd\_fric(traits, sp\_com, stand = FALSE)^*$

  > **traits** = an individual-by-individual distance matrix computed from traits. NAs not allowed.
  >
  > **sp_com** = matrix containing the abundances of the individuals of a species in traits (or presence, e.g., 1). Rows are N treatments and individuals are columns. The number of individuals of a species in sp_com cannot be less than the number of provided traits in traits, therefore FRic was unable to be calculated (equal to NA) in 2.5 N and 20 N species datasets.

stand = standardizes FRic values and scales FRic between 0 and 1 (default: FALSE).

*Code modified from Grenié and Gruson (2021) example using fd_fric function in the fundiversity (version 0.2.1.9) package.