

**Effects of nutrient addition on plant community composition: a functional trait analysis in a long-term experiment**

by

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The effects of nutrient availability on plant community composition and diversity have been well-documented, but the mechanisms behind the community response remain unclear. Plant species interact with variation in the environment through a suite of morphological, biochemical, and physiological traits known as functional traits. Analysis of functional traits can provide insights into the resource use strategies that allow plants to be successful in different environments. At two ends of a spectrum, species may exhibit conservative or exploitative strategies that differ in the rates at which they acquire and invest resources in structures and functions. Some functional traits have been shown to be related to resource use strategy. Additionally, functional traits can exhibit phenotypic response to changes in the environmental factors. The degree of phenotypic response may be ecologically important and relate to resource strategy, with exploitative species expected to have higher amounts of phenotypic response. This study, which takes place at a long-term experiment in a protected wetland site, examined eight functional traits of plant species, building upon the previously collected community data from the past 14 years. The long-term experiment was set up to study the effects of nutrient addition (fertilization) and disturbance (mowing) on plant community composition. The design, a 2x2 factorial, replicates fertilization and mowing treatments on eight blocks. A drainage ditch is also

present and runs along one edge of the experimental array. Functional trait data were collected on 46 of the most common species at the site from plants in mowed/fertilized and mowed/unfertilized plots. Functional traits from three categories were sampled: leaf traits, leaf nutrient traits, and plant size traits. Data on species abundance and functional traits were integrated to calculate community-weighted trait means to provide insight into the mechanism behind changes in community composition due nutrient enrichment. Consistent with previous studies, our results showed that, in addition to the documented species composition differences between treatments, trait composition of the plots was different between fertilized and unfertilized plots. We found that mean community trait values in the fertilized plots were shifted in the direction expected for an exploitative resource use and acquisition strategy. We also found that more conservative trait values were present in the wetter plots found farther away from, and presumably less well drained by, the ditch. Traits and species varied in their amount of intraspecific variation, and overall trait composition was heavily influenced by phenotypic response. On average, phenotypic response to fertilization was in the direction expected of exploitative species. Our results suggest that community assembly in the long-term experiment is influenced by an environmental filter for species that exhibit exploitative traits or express such traits in response to fertilization. In contrast, we found no significant relationship across species between effect size of response in abundance to fertilization and mean trait values. We found no support for the hypothesis that species with high amounts of phenotypic response were more dominant in the fertilized plots or that species with an exploitative strategy exhibit higher amounts of phenotypic response. These results have implications for predicating how species and trait composition will change in response to anthropogenic influences on nutrient cycling and deposition to the environment.



EFFECTS OF NUTRIENT ADDITION ON PLANT COMMUNITY COMPOSITION: A  
FUNCTIONAL TRAIT ANALYSIS IN A LONG-TERM EXPERIMENT

A Thesis

Presented To the Faculty of the Department of Biology

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## INTRODUCTION

Understanding how communities assemble is a major goal of ecology. The composition of communities, groups of two or more species that occur in the same space at the same time, depends on many abiotic factors, such as access to water, nutrients, and light, as well as levels of disturbance (Fukami and Nakajima 2011, Douma et al. 2012). In addition, biotic interactions, such as competition, mutualism, facilitation, parasitism, and predation, play an important role in community composition (Callaghan 1995, Brooker and Callaghan 1998, Fedriani et al. 2013, Keller and Lau 2018). Interactions among individuals of the same species (intraspecific competition) and interactions between individuals of different species (interspecific competition) have been shown to shape plant community composition (Aerts 1999, Wang et al. 2015). Competition can also affect community diversity (Wang et al. 2015).

One of the most important parameters associated with community composition is diversity, the number and relative abundance of species found in an area. Diversity can play an important role in ecosystem functioning. Communities with high diversity have been shown to have higher productivity, be more resistant to invasive species, and are more likely to withstand or recover from natural disasters and climate change (Loreau et al. 2001, Mason et al. 2005, Isabel et al. 2015). Loss of diversity can also lead to predictable changes in ecosystem functioning as species with certain traits are replaced by those with a different set of traits (Loreau et al. 2001).

Nutrient availability is known to affect plant diversity and community composition (Roem et al. 2002, Douma et al. 2012, Soons et al. 2017). The resource-ratio hypothesis gives a possible mechanism behind how nutrient availability can shape community assembly (Tilman 1982). When a nutrient is limited, plant species must compete for the resource. The minimum

amount of that limited nutrient that a plant can use to survive,  $R^*$ , determines how successful a species will be in any given environment. Environments with different nutrient concentrations select for species with different competitive strategies (Craine 2005). For example, an environment with low nitrogen concentration will tend to select for species that better compete for nitrogen. Conversely, high nitrogen environments will tend to select for species that compete more effectively for other resources, like light (Craine 2005). In a fertilization experiment in an old-field plant community, Goldberg and Miller (1990), found the addition of nitrogen significantly decreased species diversity. Before its addition, nitrogen was the limiting resource; after treatment, earlier canopy closure caused light to be limiting, which resulted in a loss of diversity (Goldberg and Miller 1990). The links between fertilization, light limitation, and diversity have also been demonstrated in a global study. In an experiment replicated in 40 grasslands on six continents, the addition of nutrients consistently reduced diversity through the increased competition for light; herbivory rescued diversity by alleviating the increased competition for light (Borer et al. 2014).

In addition to changes in diversity, differences in nutrient availability can also cause changes in community composition. In a review of the effects of nutrient enrichment, DiTommaso and Aarssen (1989) found that nitrogen addition generally increased the competitive advantage of grass species over forbs, especially leguminous species (DiTommaso and Aarssen 1989). For example, the application of a slow-release fertilizer in a serpentine grassland increased aboveground biomass but shifted plant community composition from a forb-dominated to a grass-dominated species composition (Hobbs et al. 1988). In contrast, in a study of decade-long nutrient enrichment in a tallgrass prairie, Avolio et al. (2014) found that the addition of nitrogen and phosphorus resulted eventually in greater dominance of forbs and reduced

abundance of grasses, particularly C<sub>4</sub> grass species. Surprisingly, changes in community composition resulted in no overall effects of species diversity in this study (Avolio et al. 2014). In the Park Grass study, a long-running fertilization experiment in a grassland habitat in lowland England, the application of fertilizer, which led to different interspecific competition, has eliminated species that employ a slow-growth strategy with later flowering periods (Tofts and Silvertown 2000).

The addition of nitrogen to the environment is increasingly threatening global biodiversity and ecosystem functioning (Phoenix et al. 2012). Predictions on atmospheric nitrogen deposition rates based on global chemistry transport models estimate that, by the year 2050, rates of N deposition could more than double the rates found in the mid-1990s (Phoenix et al. 2006). The addition of other nutrients, like phosphorus, can also impact plant communities. In a survey analysis of 640 studies in which nitrogen and phosphorus were added to freshwater, terrestrial, and marine systems, Harpole et al. (2011) found that, in over half the studies, communities displayed a synergistic response to the addition of these nutrients. Furthermore, communities only responded when both nitrogen and phosphorus were added in 28% of the total 641 studies. Nutrient availability varies across communities naturally, however, human activities can also influence nutrient concentrations. Agricultural fertilizer run-off and nutrient deposition from industrial pollution or the burning of fossil fuels can add nutrients to natural habitats with documented effects on plant community diversity, composition, and function (Vitousek et al. 1997, Bobbink et al. 2010). Indeed, more N is fixed by anthropogenic processes than by natural ones (Vitousek et al. 1997). The addition of nutrients can also increase a habitat's susceptibility to invasive species, and nutrient enrichment can also increase an invasive species' "invasiveness" (Perry et al. 2004, Uddin and Robinson 2018). Wetland plant communities,

which are typically N limited (Perry et al. 2004), may be particularly susceptible to plant invasions following nutrient addition. These dramatic responses in community structure argue for better understanding of the mechanisms behind the changes in communities due to anthropogenic nutrient enrichment.

Members of ecological communities interact with their environment through a suite of characteristics known as functional traits. These traits can be morphological, biochemical, or physiological (Díaz et al. 2013). Functional trait approaches revolutionized the study of community ecology starting in the early 1990s (Chapin et al. 1996). The concept of functional traits builds upon an older literature that looks at plant species and the traits that determine their survival strategies. The universal adaptive strategy or CSR triangle hypothesis (which includes competitors, stress-tolerators, and ruderals) of plant ecology, which describes the combined effects of stress and disturbance on plant community composition (Grime 1979), has been influential in plant community ecology (Craine 2005; Pierce et al. 2013). Since the 1990s, increasing focus on functional traits has shifted community ecology from an emphasis on species and their relative abundances to the biological characteristics of those species (Ali et al. 2017).

Investigation of functional traits can provide insights into fundamental processes in ecology. For example, trait studies have been used to evaluate two major contrasting theories of community assembly: competition theory and the environmental structuring hypothesis (Tofts and Silvertown 2000). The competition theory, or idea of limiting similarity, predicts that trait values of species that coexist in a community should be more different than expected by chance. Traits are expected to be more different because more similar species are also expected to occupy similar niches and therefore to compete more strongly for resources. Ecological theory states that no two species can occupy the same niche in the environment at the same time (Gause

1930), which causes a divergence in traits between the species (Grime 2006, Godoy et al. 2014). The environmental structuring or filtering hypothesis predicts that traits of species in a community should be more similar than expected by chance. This hypothesis explains how environmental factors may act to select for certain functional traits or trait values. Not all species are able to establish and survive in all environmental conditions. Thus, individuals with certain traits are more successful under certain biotic and abiotic constraints (Lebrija-Trejos et al. 2010, Kergunteuil et al. 2018). This acts to create a filter by which environmental conditions select for species with traits that are most competitive in those conditions. Some studies (Kraft et al. 2015) have shown that stressful conditions related to abiotic factors in the environment can lead to trait convergence through environmental filtering.

The diversity of the functional trait values found in species in a community can be quantified and used to distinguish between these. Competition theory predicts that a community will have high functional diversity, which is due to a divergence in trait composition in response to competition. The environmental structuring hypothesis would predict the opposite: functional diversity should be low, since the environmental filter has homogenized traits or trait values (Lambers et al. 2010). In reality, both of these ecological hypotheses both may help explain what is happening in the community. In a study of traits in a wetland plant community, Weiher et al. (1998) found a reconciliation of these two principles. Their results showed that abiotic conditions constrained certain traits (within limits), while biotic forces kept coexisting species from becoming too similar.

Functional traits can provide information on the mechanisms by which nutrient availability impacts community assembly (Douma et al. 2012). Traits that affect resource use and acquisition are particularly relevant. Plant resource strategies can be broadly characterized along

a spectrum from conservative to exploitative (Chapin et al. 1996, Grassein et al. 2010, Alvarez-Yepiz et al. 2017). Conservative species, much like the stress-tolerant species from Grime's CSR scheme, are adapted to environments where nutrients are limited. Species with a nutrient-conservative strategy invest acquired resources in long-lived, relatively expensive structures and exhibit slow growth patterns (Grassein et al. 2010). They are expected to show high leaf dry matter content (ratio of dry weight to wet weight) and low specific leaf area (ratio of leaf area to dry mass), a related measure of leaf thickness as well as cellular and tissue construction. Conservative plants show slow turn-over of these expensive leaves (the rate at which plants lose and replace their leaves). At the other end of the spectrum, exploitative species are especially adept at taking up nutrients from environments where they are readily available for rapid growth and production of cheaper structures (Grassein et al. 2010). Exploitative species have high leaf turn-over, low leaf dry matter content, and high specific leaf area. Leaf nitrogen content, a measure of how well the root system of a plant can take up and distribute nutrients, is generally high in exploitative species (Grassein et al. 2015). A related concept that is central to plant ecology is the leaf economic spectrum, or how plants invest and re-invest carbon and mineral nutrients to the leaves (Wright et al. 2004). Understanding the leaf economic spectrum can add predictive ability to how plant communities will respond to nutrient fluctuations and changes in the environment due to climate change and land usage. Functional traits associated with resource use strategies are tightly linked to ecosystem functions, including productivity and litter decomposition (Quetier et al. 2007). Understanding how nutrient levels affect trait composition can help to predict responses in both the species composition of communities and the services they provide.



In much of the literature, functional traits are treated as fixed species-level entities (Ackerly and Cornwell 2007); however, traits show considerable intraspecific variation, and this variation may be ecologically important (Funk et al. 2007). Some intraspecific variation is caused by phenotypic plasticity, which refers to an organism's ability to respond to its environment through a change in morphology, physiology, or behavior without a change in genotype (Via et al. 1995, Callaway et al. 2003, Pigliucci 2005, Born and Michalski 2017). The ability to respond to the environment is especially important for plants because they are sessile. Plasticity itself is a trait that can be measured, can evolve, and can add or detract from an individual's fitness (Nicotra et al. 2007). Plasticity in functional traits can be responses to abiotic factors, such as water, light, and nutrient availability, as well as to biotic influence. Neighbors within the community, including conspecific individuals, can influence trait expression (Abakumova et al. 2016). The level of plasticity of a species is expected to correlate positively with its niche breadth (Pohlman et al. 2005); however, support for this idea has been mixed (Dostál et al. 2016). Niche breadth, or the range of resources a species uses, may determine geographic distribution (Slatyer et al. 2013); phenotypic plasticity may serve to increase ability of a species to adapt to a wider range of habitats with varying resources. Much of the study of plasticity in functional traits has addressed invasive plant species with a goal of understanding how phenotypic response contributes to their success as an invader (Zou et al. 2007, Huang et al. 2016). For example, Huang et al. (2016) found that two invasive plant species showed more plasticity in response to nutrient addition than the native species, specifically in SLA, and that plasticity may contribute to invasiveness.

Trait plasticity, and how this plasticity affects community composition, has received increasing interest in the study of functional traits (Valladares et al. 2006, Funk et al. 2007,

Nicotra et al. 2007). In those functional trait studies where individual variation is measured, trait shifts in response to environmental factors by individuals have been found to contribute substantially to overall community trait variation along environmental gradients for some traits and levels of sampling (Jung et al. 2010, Auger and Shipley 2013, Carlucci et al. 2015). Furthermore, plasticity in response to nutrient addition can affect the abundance of plant species, at least to some degree (Firn et al. 2012, Grainger and Turkington 2013, Dostál et al. 2016). For example, in a fertilization study of boreal forest understory species, *Epilobium angustifolium* showed the greatest morphological response to fertilization and also showed the greatest increase in abundance with treatment (Grainger and Turkington 2013). Exploitative species have been shown to have higher plasticity in their functional traits and their amount of productivity following higher nutrient treatments. In a study of two sub-alpine species, an exploitative species (*Dactylis glomerata*) had an overall higher level of plasticity, as well as higher productivity with the addition of nutrients (Grassein et al. 2010). Studying the plasticity levels of functional traits, and how that plasticity evolves (Schlichting 1986), can provide insight into plant community assembly.

We used a trait-based approach to study plant community assembly and resource strategies in a long-term ecological experiment at a disturbed wetland site in eastern North Carolina (Goodwillie and Franch 2006). Wetlands are delicate ecosystems that play a critical role in the environment, therefore, understanding of the response to nutrients in wetland plant communities is critically important. Natural wetlands are sometimes nutrient-poor environments where few plant species, except stress-tolerant individuals, are successful. Wetlands make up about 6% of the world's land mass, yet they store around 12% of the world's carbon (Erwin 2009). In addition to carbon storage, wetlands can also serve as a storage site for excess

nutrients, such as nitrogen, which are added to the environment by humans through industrial processes or fertilizers run-off (Born and Michalski 2017). The storage of these excess nutrients can cause a drastic change in community composition due to the removal of the competitive advantage for the stress-tolerant species (Grime 1979).

Since 2004, we have documented dramatic effects of fertilization treatments on the plant community in the long-term experiment. Not only has community composition changed in response to nutrient addition, but a loss of diversity has also occurred through time. In the study presented here, we integrated functional trait data with the long-term abundance data to give insights into the mechanisms behind these community responses. Species abundance and functional traits were combined to explore how community mean trait values differed between fertilized and unfertilized plots. Community-weighted trait means provide a way of observing trait means in differing environmental states. This is done by weighting a species' relative abundance by its trait value (Garnier et al. 2004).

We also quantified intraspecific variation of functional traits in response to fertilization by measuring traits separately in fertilized and unfertilized plots to provide information on how phenotypic plasticity contributes to variation in community composition. Although species trait shifts between treatments could also be due to genetic differences in individuals found in the fertilized and unfertilized plots, we argue that evolutionary changes are unlikely to have occurred in the short time scale and small spatial scale of the experiment. As a result, we focus on the implications of species trait shifts primarily in terms of plasticity but discuss other possible explanations below.

We tested the hypothesis that traits that are associated with resource use and acquisition differ between fertilized and unfertilized plots. We used community-weighted trait means, a

composite measure of species trait values weighted by their abundance, as a response variable. We expected that species exhibiting more exploitative trait values would be favored in the nutrient-enriched plots; conversely, we expected species with more conservative trait values to be more abundant in unfertilized plots. Thus, we expected our CWTM values would be more exploitative in fertilized plots and CWTM values in unfertilized plots to be more conservative. We also quantified differences in species trait values between plants collected in fertilized and unfertilized plots. Based on previous research, we expected that species with a more exploitative resource strategy to also exhibit higher phenotypic response to nutrient addition. We hypothesized that species with higher levels of phenotypic response would be more abundant in the fertilized treatment. We also considered the hypothesis that, if plasticity increases the niche breadth of a species, species with higher levels of phenotypic response would be equally abundant in both fertilized and unfertilized treatments.

## MATERIALS AND METHODS

### Study Site

The long-term experiment is located at the East Carolina University West Research Campus (WRC), which was formerly a Voice of America site. The WRC is a 235 ha site found in the central coastal plain of North Carolina. Located at one of the highest points in Pitt County, the WRC sits at a 22-25 m elevation. The soil is poorly drained, partially due to the location of the site between the Neuse and Tar rivers. Six soil types, some of which are poorly draining soils, have been found at the site including Coxville, Lynchburg, Goldsboro, Rains, Exum, and Bibb (Chester 2004). Based on similarity to reference plant communities, Chester (2004) hypothesized that the site was once a combination of pine savannah, wet pine flatwood, and hardwood forest plant communities before human intervention. For example, species found in these habitats may include *Arundinaria tecta*, *Chasmanthium laxum*, *Pinus taeda*, *Liquidambar styraciflua*, *Acer rubrum*, as well as species found in the *Solidago* and *Eupatorium* genera.

### Experimental Design

The long-term experiment, for which data have been collected each year starting in 2004, is replicated on eight blocks measuring 20 by 30 m (Figure 1). Each block is then divided into four treatment plots. Mowing (disturbance) and fertilization treatments are applied in a 2x2 factorial design to yield four plot types: mowed, fertilized, mowed and fertilized, and control (unmowed, unfertilized). Within each treatment plot, three-1 m<sup>2</sup> sampling quadrats are randomly located. The quadrats are placed at least 2 m from the perimeter of each plot to prevent edge effects and avoid potentially confounding effects of fertilizer run-off. Pellet fertilizer has been applied to the fertilized treatments (fertilized and mowed/fertilized) three times a year in February, June, and October. The mowed and mowed/fertilized treatments are mowed and raked

of debris once a year in February. In early August, undergraduate students collect plant community data from the permanent quadrats. They record both stem count and percent cover data for all plant species found within each 1 m<sup>2</sup> quadrat for the following variables: 1) total plant density (number of stems for all species present in each quadrat) and 2) percent cover as a measure of the structural dominance of a species. A ditch is present adjacent to the study site and runs alongside the odd-numbered blocks at the site. Drainage by the ditch appears to cause a moisture gradient, with blocks near the ditch drier than blocks away from the ditch (C. Goodwillie, unpublished data).

### **Functional Trait Measurements**

Eight functional traits were chosen for use in this study because they are known to be associated with variation in resource use and acquisition strategies and leaf economics, including leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC), leaf carbon content (LCC), leaf carbon-nitrogen ratio (CNR), final plant height (FH), and final aboveground biomass (FB) (Figure 2). The effects of fertilizer were the focus of this study, so only data for mowed plots were used. Mowed, rather than unmowed, plots were used because, historically, this community is thought to have had regular disturbance events such as wildfires, which are replicated by the mowing treatment. Functional traits were measured for the 46 most common species (Table 1) found in mowed plots. To the extent possible, samples used for trait measurement were distributed evenly among blocks; however, some species are not present in all blocks. Rigorous randomization was not feasible in sample collection; however, plants were selected of a size that was representative of most plants in the plot to avoid sampling bias.

Plant Size Traits: Data for final height (FH) and final aboveground biomass (FB) were collected in late August, near the end of the growing season. We selected five stems of each

species from both the mowed and the mowed/fertilized treatments by clipping at ground level. We measured height of sampled plants, then oven dried and weighed them to give final aboveground biomass.

Leaf Traits: Collection of the leaves (used to calculate leaf area, specific leaf area, leaf dry matter content, as well as leaf nutrient traits) occurred during peak growing season from mid-May until early September, with earlier flowering and fruiting species collected first. Leaves were collected from individuals in both mowed and mowed/fertilized plots, when possible. A few species were rarely found in the mowed/unfertilized plots, and samples for these species were supplemented with plants found in surrounding areas where fertilizer was not added (Table 1). Samples of some woody species were collected from the unmowed treatment plots (control and fertilized) (Table 1). Leaves from 16 plants of each species were collected from each treatment type when possible (mean = 13.5); in a number of cases, it was not possible to collect 16 samples because of low abundance of some species (lowest sample size = 5) (Table 3).

Selection of leaves for harvest differed by species but was standardized within species. In species with a terminal inflorescence, leaves were collected at a specified position by counting nodes downward from the inflorescence. In species without a terminal inflorescence, leaves were collected by counting from the tip of the branch to a specified leaf position, which varied among species. Collected leaves were placed in sealed plastic bags with a damp paper towel and placed into a cooler with ice to avoid desiccation. Fresh leaf samples were weighed immediately after sampling, taped to newspaper and labeled, pressed, oven dried for 48 hr, and then reweighed. Leaf dry matter content (LDMC) was calculated for each leaf as dry weight/wet weight. Leaves then were photographed to determine leaf area using Photoshop (2015.5 version 17.0.2). Specific leaf area was then calculated as leaf area/dry weight. For leaf nitrogen and carbon content

analysis, small pieces of dried leaf samples were pulverized using mortars and pestles after freezing with liquid nitrogen. Small pieces of each leaf collected within a treatment (fertilized or unfertilized) were combined to give a value that was representative of the population. We measured total nitrogen and carbon content of leaves using the combustion method with the Perkin Elmer 2400 CHNS Analyzer. North Carolina State University Environmental and Agricultural Testing Service (EATS) laboratory (<http://www.soil.ncsu.edu/services/asl/index.html>) provided elemental analysis.

## **Analyses**

Species Composition Analysis: Species abundance data and functional trait measurements in fertilized and unfertilized plots were used both separately and together to test hypotheses on community assembly. See Figure 3 for an overview of analyses and research questions. We derived a matrix that described pairwise differences in the species composition of treatment plots based on Bray-Curtis dissimilarity using data from the 2017 season. Species abundance was quantified using importance values (IV), which were calculated as the sum of relative stem count (stem count of a species/total stem count) and relative percent cover (percent cover of a species/total percent cover) in each 1 m<sup>2</sup> quadrat.

We used the Bray-Curtis dissimilarity matrix for permutational multivariate analysis of variance (PERMANOVA) to test for effects of fertilization, proximity to ditch, and block on multivariate species abundance in the plant community. Fertilization and ditch were treated as fixed factors, while block was designated as a random factor. Block was nested within the ditch factor for the analysis. The analysis was done with permutations of residuals under a reduced model, with permutation number set at 999. The sum of squares for the model was type III (partial), and the fixed effects summed to zero. Principal coordinate analysis (PCO) was used



with the Bray-Curtis matrix to visualize multivariate data on species composition. The maximum number of principal components was set to 5. PERMANOVA and PCO were completed in Primer (vers 6.1.13, Clarke 2006).

Phenotypic Response Analyses: To test the hypothesis that exploitative species would exhibit a higher degree of phenotypic response, we used Spearman's rank correlation to test for an association between magnitude of phenotypic response and the mean unfertilized trait value for each trait. The magnitude of phenotypic response was quantified as the log response ratio of fertilized trait value to unfertilized trait value (Hedges et al. 1999). Significance values were adjusted at the table-wise level using sequential Bonferroni procedure (Rice 1989). Correlation analyses were completed in SPSS 25 (IBM 2017).

Trait Composition Analyses: To explore the data, we tested for correlations between the mean fertilized trait values for all traits using Spearman's rank correlation across all species. Significance values were adjusted at the table-wise level using sequential Bonferroni procedure (Rice 1989). Community-weighted trait means (CWTM) were used to quantify overall trait composition in fertilized and unfertilized plots. For each of the eight traits, the CWTM was calculated in each quadrat as the sum across all species of each species' importance value multiplied by its mean trait value. CWTM values were calculated in two ways. Analyses were first done using a fixed-species trait value for all quadrats. The trait value in unfertilized plots was used as the fixed-species trait value because it represents the unmanipulated condition of the species at this site. Analyses using CWTM values from the fixed-species trait value account only for differences in mean community trait composition due to variation in species composition. Analyses were then done using treatment-specific trait values; that is, trait means from fertilized plots were used to calculate CWTM values for fertilized quadrats and trait means from

unfertilized plots were used to calculate CWTM values for unfertilized quadrats. The treatment-specific analyses measure differences in mean community trait composition due to both variation in species composition and phenotypic response to fertilization in individual species.

A resemblance matrix was made of pairwise differences between treatment plots in community weighted mean trait values based on Bray-Curtis dissimilarity. A PERMANOVA was used with the matrix data to test for overall differences between fertilized and unfertilized plots in trait composition across all traits. This was done using the two types of CWTM: fixed-species trait value and treatment-specific trait values. The PERMANOVA tested for effects of fertilization, proximity to ditch, and block on community-weighted trait means in the plant community. Fertilization and ditch were treated as fixed factors, while block was designated as a random factor, which was nested within the ditch factor. The analysis was done with permutations of residuals under a reduced model, with permutation number set at 999. The sum of squares for the model was type III (partial), and the fixed effects summed to zero. Principal Coordinate Analysis (PCO) was then used to visualize the multivariate data using the Bray-Curtis matrix. The maximum number of principal components was set to 5. Vectors were added to the PCOs to visualize how trait values were correlated with each principle component axis.

Analysis of variance (ANOVA) was used to determine the effects of fertilizer and ditch on CWTM values for individual traits. As in the multivariate approach, ANOVAs were done using both fixed-species trait CWTM values and treatment-specific CWTM values. The model included proximity to ditch and fertilization as fixed factors and block as a random factor nested with ditch. ANOVAs were completed using SPSS 25 (IBM 2017).

To test whether species with exploitative trait values are more successful in fertilized treatments, changes in species abundance in response to fertilization were correlated with mean

fertilized trait value across all species studied using a Spearman's rank correlation. Effect size was used to quantify the magnitude of the response in species abundance for the correlation analyses. Effect size was calculated as the difference in means between the unfertilized and fertilized treatments divided by the standard deviation. To test the hypothesis that species with higher phenotypic response are more successful in the fertilization treatment, changes in species abundance were correlated (Spearman's rank correlation) with the magnitude of phenotypic response. Again, effect size was used as the measure of change in abundance in response to fertilization, while phenotypic response was quantified as the log response ratio of fertilized to unfertilized trait value. Both correlation analyses were carried for each of the traits in SPSS 25 (IBM 2017).

## RESULTS

### Species Composition Analysis

PERMANOVA results indicated that species composition differed between fertilized and unfertilized plots. Species composition was not significantly different among blocks (Table 2). Our results showed a significant effect of proximity to the ditch on species composition, which is consistent with analysis of the long-term data that suggests a highly significant effect of the ditch on community composition (C. Goodwillie, unpublished results). The PCO plot (Figure 4) showed strong separation of fertilized and unfertilized plots in species composition, primarily along the first axis, which explained 46.9% of the variation. Plots were also separated along both axes according to their proximity to the ditch, though the separation was not as dramatic compared to separation by treatment. The second axis represented 13.2% of the total variation. Groups appeared to cluster together: fertilized plots were more similar to each other than to unfertilized plots. A similar pattern was observed between wetter and drier plots. Fertilization and drainage by the ditch appear to drive the community composition in a similar direction.

### Phenotypic Response Analyses

We examined phenotypic response to fertilization in all species using percent difference between fertilized and unfertilized trait values and log response ratio of fertilized to unfertilized trait value for each trait. We found that most species exhibited trait shifts in response to fertilization (Table 3). Most species exhibited phenotypic response in two to three traits, and 14 species showed high plasticity, exhibiting responses in four or five traits. For example, members of the *Eupatorium* genus generally exhibited high amounts of plasticity in functional traits. Final height had the highest amount of species exhibiting phenotypic response, despite the lower sample sizes compared to the leaf traits. Final biomass had the lowest amount of species

exhibiting phenotypic response. For some traits, species showed responses to fertilization in opposite directions (Table 3). In most comparisons, however, fertilized trait values were shifted in the direction expected for more exploitative traits (Figure 2), which are expected to be common in high nutrient environments.

### **Multivariate Trait Composition Analyses**

The PERMANOVA was run using the fixed-species trait value (unfertilized) for the CWTMs, then again using the treatment-specific trait value for the CWTMs. For both PERMANOVAs, none of the interaction terms or the block term yielded a significant result. For both analyses, the mean trait composition differed between fertilized and unfertilized plots as well as those plots near or away from the ditch (Table 2). PCO for community-weighted trait values showed clustering of plots by fertilization and proximity to ditch (Figure 5). Effects on trait shifts were more dramatic in the analyses that included phenotypic plasticity (treatment-specific CWTM) (Figure 6, Table 2). Eigenvectors were calculated for each axis to determine how much each trait contributed to the separation of plots. For the fixed-species trait value analysis, the first axis (PCO1), which accounted for 47.6% of the total variation, was associated with five traits: LA, SLA, LNC, and CNR. Eigenvectors were calculated at -0.8071, -0.6794, -0.7941, and -0.6559, respectively. Thus, fertilized plots had higher leaf area, specific leaf area, leaf nitrogen content, and carbon-nitrogen ratio. Axis 2 (PCO2), accounting for 32.1% of the variation, was primarily driven by three traits (LDMC, LCC and FB) with eigenvector values of -0.5876, -0.8929, and 0.7274. Thus, wetter plots had higher LDMC and LCC and lower FB trait values. For the treatment-specific trait value analysis, all traits, except LCC, had eigenvector values with absolute values greater than 0.5. For PCO1, which accounted for 72.7% of the variation, all were positive eigenvector values except for LDMC and LCC, which follows the

expectation for exploitative species dominating fertilized plots. Thus, drier and fertilized plots were dominated by plants with lower leaf dry matter content and higher trait values in leaf area, specific leaf area, leaf nitrogen content, carbon-nitrogen ratio, and final height and biomass. PCO2 also had several traits contributing to the differentiation and explained 17.9% of the total variation. CNR and LCC had eigenvector values with absolute values greater than 0.5, indicating that fertilized plots had lower leaf carbon content and higher carbon-nitrogen ratio.

### **Individual Trait Analyses**

When analysis of variance was done using the fixed-species CWTM, which tested for differences only in species composition, mean trait values differed significantly between fertilized and unfertilized plots for LA and SLA, but not LDMC (Table 4). LA and SLA were also significantly different in relation to proximity to the ditch. SLA was the only leaf trait that varied significantly among blocks. Interestingly, LDMC had a significant interaction between fertilizer and block nested in ditch. Mean LA was 24% higher in fertilized plots, which follows the expected trend for species with an exploitative resource strategy. Mean SLA, however, was 5% lower in fertilized plots counter to expectations (Figure 7). Mean LA was also 47% larger in the plots close to the ditch, which is the direction expected for exploitative species (Table 5). Use of treatment-specific trait values in the analysis takes into account both species composition and phenotypic responses within species. In this analysis, LA and SLA significantly varied between fertilized and unfertilized plots; LDMC trended toward differing between treatment plots ( $P = 0.051$ ). All three leaf traits were significantly different between plots near and far from the ditch. Additionally, the magnitude of the differences in the treatment-specific analysis were greater than in the fixed-species trait analysis (Figure 7). Mean LA and SLA were 96% and 7% higher in fertilized plots, respectively; mean LDMC was 3% smaller in fertilized plots (Figure 7). Mean

LA and SLA both had higher trait values in plots closer to the ditch with an increase of 55% and 9%, respectively (Table 5). Mean LDMC had 6% decrease in plots near the ditch. Again, SLA showed variation among blocks. None of the interaction terms were significant in the treatment-specific analysis.

For the leaf nutrient traits in the fixed-species trait value analysis, mean LNC and CNR were significantly different between fertilized and unfertilized plots (Table 4). Mean LNC exhibited a 10% increase in the fertilized plots which follows the expected trend of exploitative species (Figure 8). Mean CNR increased by 18%, which does not follow the expected trend for exploitative species (Figure 8). Only LNC significantly differed in relation to ditch proximity, with a 22% increase in plots near the ditch (Table 5). Only LNC trended toward differing by block ( $P = 0.054$ ). Interestingly, LCC had a significant interaction between fertilization and block nested in ditch. When treatment-specific trait values were used, LNC and LCC were not significantly affected by fertilization treatment, while CNR remained significant and increased in fertilized plots by 2% (Table 6, Figure 8). LNC significantly differed in relation to proximity to the ditch (a 17% increase in plots near the ditch), while the other two were not significant. Both LNC and CNR were different among blocks. LCC remained significant for the interaction of fertilization and block nested in ditch when using treatment-specific values (Table 6).

For the plant size traits, FH was significantly different between fertilized and unfertilized plots when using fixed-species trait values in the analysis (Table 4). FB, however, trended toward differing between fertilized and unfertilized plots ( $P = 0.065$ ). Consistent with the hypothesis that high nutrients select for species with exploitative traits, mean FB was 32% higher in fertilized than in unfertilized plots (Figure 9). Contrary to the expected pattern for exploitative species, mean FH was 7% lower in the fertilized plots. Proximity to ditch was only significant in

the FH trait, with a mean decrease of 7% in plots near the ditch (Table 5). Neither plant size trait varied significantly among blocks or displayed a significant interaction between factors. In the analysis using treatment-specific trait values, mean FH and FB were significantly higher in fertilized than unfertilized plots (Table 6), 45% and 206%, respectively, which follows the expected trend for exploitative species (Figure 9). However, only FB yielded a significant result for difference in relation to proximity to the ditch, with plots close to the ditch having 31% more biomass (Table 5). FH was significant in the interaction of fertilization and ditch proximity, with fertilization having a greater negative effect on FH in plots near the ditch. Final biomass trended toward a significant interaction between fertilizer and block (nested in ditch).

In testing the hypothesis that species with greater phenotypic plasticity were favored in fertilized plots, a Spearman's rank correlation analysis of effect size of abundance and magnitude of phenotypic response (log response ratio) yielded no significant associations (Table 7). We found only limited support for the hypothesis that species with exploitative traits were more successful in fertilized plots. Spearman's rank correlation analysis found that for only two traits, FH and FB, mean fertilized trait values were significantly correlated with the effect size of abundance (Table 7). Additionally, we found little support for the hypothesis that species with typical exploitative trait values also show a greater phenotypic response to fertilization. Spearman's rank correlation analysis yielded two traits (CNR and FH) with significant associations between mean unfertilized trait value and log response ratio of fertilized to unfertilized trait values (Table 7). There were, however, some significant correlations between mean fertilized traits values: LA was correlated with FB and LCC, SLA with LDMC, LNC, and FH, LDMC with LNC and CNR, LNC with CNR, and FH with FB and LCC (Table 8).



## DISCUSSION

In a long-term experiment in a wetland habitat, we found that nutrient addition resulted in variation in functional trait variation among plant communities associated with nutrient availability. Fertilized plots were generally composed of trait values associated with a shift towards the exploitative end of the resource use spectrum (Figure 2), while trait values associated with a shift toward the conservative end of the spectrum were more common in the unfertilized treatment. These trends, observed in both multivariate and individual trait analyses, were substantially stronger, however, when intraspecific variation was included in the analyses.

Fertilization has resulted in substantial changes in community composition in the long-term experiment. A multivariate analysis of the abundance of the 46 most common plant species at the site revealed a significant effect of the fertilization treatment on community composition, and a PCO plot showed clustering of fertilized and unfertilized plots. Our findings support those of other studies showing that nutrient addition alters plant community composition and diversity (Thurston 1968, Hobbs et al. 1988, DiTommaso and Aarssen 1989, Bobbink et al. 2010). Inspection of species effect sizes of abundance response to fertilization shows two main trends in the divergence of community structure of fertilized and unfertilized plots. Fertilized plots show an increase in upland species such as *Rhus copallinum* (winged sumac) and *Rubus argutus* (blackberry), whereas wetland specialists species, such as *Solidago stricta*, *Rhynchospora inexpansa*, and *Polygala cruciata*, show steep declines. Secondly, small herbaceous species, such as *Lobelia nuttallii*, *Rhexia mariana*, and *Polygala cruciata*, showed decreases in abundance with fertilization. While previous studies found that nutrient addition caused shifts from forb- to grass-dominated communities (Hobbs et al. 1988, DiTommaso and Aarssen 1989), our results showed that grass species responded individually, with increases in abundance (*Arudinaria tecta*

and *Chasmathium laxum*) and decreases in abundance (*Andropogon virginicus* and *Aristida virgata*).

Our results suggest that in addition to changes in plant community species composition, functional traits associated with resource use strategy are contributing to the divergence of the experimental communities based on nutrient availability. Exploitative species are characterized by short leaf lifespan, fast growth, and high nutrient uptake, and are often found dominating high nutrient habitats (Chapin et al. 1996, Grassein et al. 2010, Schellberg and Pontes 2012). Consistent with our expectations, we found that fertilized and unfertilized plots significantly differed in the abundance of plants with traits associated with resource use (Table 2). Functional traits separated the treatment plots in the direction expected, with more exploitative values found in the fertilized plots in multivariate analyses using the fixed-species trait values for each species. These fixed-species trait analyses reflect variation in traits due solely to shifts in species composition. In a PCO plot, four traits (LA, SLA, LNC, and CNR) were the primary drivers of differentiation in axis 1 based on their eigenvector values, while differentiation in the second axis was mainly driven by LDMC, LCC and FB (Figure 5). Our results show support for the idea that environmental filtering plays a role in community assembly by selecting for certain traits that allow species to be successful in given abiotic conditions (Lavorel and Garnier 2002, Lebrija-Trejos et al. 2010, Zhang et al. 2014). The addition of nutrients has been shown to affect plant community assembly and can be one mechanism that filters for species with certain traits or trait values. The dominance of exploitative species in fertilized plots may have implications for the loss of diversity with nutrient addition. Species that are able to grow rapidly and produce cheaper structures may be able to outcompete wetland specialist species that are adapted for slow growth and species of short stature that become light-limited. For example, *Eupatorium*

*rotundifolium*, *Clethra alnifolia* and *Dichantheium lucidum* all have more exploitative trait values for SLA, LDMC, and FH and also had higher abundance in the fertilized plots.

When phenotypic response was added into the analysis, we found even stronger support for community trait variation due to the fertilization treatment. In addition to the fertilization treatment filtering for species with certain traits or trait values, fertilization also caused a phenotypic response in many species, as indicated by *t*-test results. In PERMANOVA, the effects of fertilization on functional trait composition were more strongly significant when treatment-specific values were used (Table 2), which account for both differences in species composition and phenotypic responses. Furthermore, in PCO plots (Figures 5,6), the two treatment plots separated more distinctly when treatment-specific trait values were used. Separation in composition followed the expected trends for exploitative species, with all trait values increasing in the fertilized plots, except LDMC and LCC, whose values were expected to decrease with fertilization.

When compared to other studies that studied similar traits in the resource use strategy, our CWTM values sat more in the middle of the spectrum. Buzzard et al. (2016) found that in forest succession, early growth was dominated by plants exhibiting a conservative resource strategy with CWTM for SLA between 50 and 100. As succession occurred, more plants with an exploitative strategy led the CWTM for SLA to increase to a range of 150-200. Our CWTM values for fertilized plots, which showed a shift toward more exploitative trait values, had a range of 129-140. Our unfertilized plots had a range of 111-137.

Our study highlights the importance of considering intraspecific variation and phenotypic response in functional trait analyses. While functional trait approaches have contributed to our understanding of community ecology, many previous studies have assigned each species a

fixed functional trait value (Lavorel and Garnier 2002, Douma et al. 2012). Indeed, global collaborative functional trait databases have made it possible to carry out trait analyses at broad scales (Wright et al. 2004, Kattge et al. 2011). However, intraspecific variation in functional traits, particularly in response to differing environmental gradients, can affect overall species trait values (Via et al. 1995, Callaway et al. 2003, Nicotra et al. 2007). Thus, accounting for intraspecific variation is critical for accurate predictions and modeling of changing plant communities in response to these constantly changing factors. Phenotypic response specifically to nutrient availability has been shown to contribute to overall functional trait variation (Firn et al. 2012, Dostal et al. 2016, Huang et al. 2016, Fajardo and Siefert 2018). In a study of sapling leaf economic traits in a temperate rainforest, Fajardo and Siefert (2018), found that intraspecific variation across soil nutrient gradients contributed to community trait variation. As in our study, they found that the direction of shifts caused by phenotypic response were congruent with community trait shifts caused by species composition; both shifts were in the direction expected of resource strategy.

Consideration of results from individual trait analyses provides further insight in environmental filtering and community assembly. When the eight traits were analyzed individually, we found mixed support for the hypothesis that more exploitative traits were favored with fertilization. We examined three leaf traits that have been found to be associated with the resource use spectrum: LA, SLA, and LDMC. Leaf area (LA) and specific leaf area (SLA), which is defined as the leaf area divided by the dry mass, are indicators of the photosynthetic ability of a plant (Cassia-Silva et al. 2017). SLA, which is related to leaf thickness, is also a measure of resource acquisitive ability and investment in plant structures, with high values indicating a low resource investment; as a result, species that exploit resources

rapidly in high nutrient environments are thought to have high LA and SLA values (Poorter and Bongers 2006). Consistent with other studies (Takarto and Knops 2018), we found that mean LA of the plant community was higher in the fertilized plots than unfertilized plots. The differences in mean trait value were evident in analyses with and without phenotypic response, though the effect was much more dramatic when accounting for plasticity. Phenotypic response to fertilization changed the mean trait value in the direction of the exploitative end of the resource spectrum. LA displayed a high degree of phenotypic response, which, averaged across species, increased leaf size by 25%. In contrast, SLA showed the opposite pattern when phenotypic response was not added in the analysis. In ANOVA using fixed-species trait values, mean SLA was significantly lower in fertilized plant communities. However, when treatment-specific trait values were used, mean SLA was higher in fertilized plots, suggesting an important contribution of phenotypic response to trait variation. SLA also showed phenotypic response in the direction of exploitative species, although the mean degree of plasticity was lower at only 7%.

Leaf dry matter content (LDMC), which is defined as the difference in wet and dry leaf mass, gives a measure of a species' investment in structures (Grassein et al. 2015). As water content increases, the dry matter of the leaf decreases, indicating low investment in structures. Exploitative species are thought to produce cheap, easy-to-manufacture structures, so they are expected to have a low trait value for LDMC. Following with previous studies (Grassein et al. 2015), mean LDMC values were lower in the fertilized plots than unfertilized plots. We also found very little phenotypic response, with a mean trait shift of only 4%, though the direction of plasticity was in the expected direction for exploitative species.

We also examined two plant size traits: final height (FH) and final biomass (FB). Similar to LA and SLA, these growth traits, especially FH, increase to aid a species in competing for

light, which is thought to become the limiting resource as nutrients become unlimited. FH allows a species to grow tall enough to reach light before other less competitive species (Cassia-Silva et al. 2017) and FB allows for out-shading those less competitive species (Grainger and Turkington 2013). Species that can exploit high nutrient environments are thought to have high trait values for both height and biomass, as these species are able to quickly take up and use nutrients. As in previous studies (Grainger and Turkington 2013), we found both FH and FB to be larger in the fertilized plots. These two traits also exhibited the highest amounts of phenotypic response, with height and biomass increasing in fertilized plots by a mean of 43% and 102%, respectively. This followed the direction expected for exploitative species. The large phenotypic response in whole plant size traits suggests that the plant community is strongly nutrient limited, a finding that is consistent with generally low fertility of wetland soils (Suter and Edwards 2013).

The final three traits examined in this study were leaf nutrient traits, which included leaf nitrogen and carbon content (LNC, LCC) and carbon-nitrogen ratio (CNR). LNC is an indicator of a species ability to take up and use resources (Cassia-Silva et al. 2017) and photosynthetic ability (Jin et al. 2014). LCC, however, also provides insight into water-use efficiency and plant growth (Cassia-Silva et al. 2017). CNR is a measure of leaf quality, which is dependent on resource availability and uptake (Mitchell et al. 2017). As a result, species adept at taking up and using resources are expected to have high LNC but low LCC (Mitchell et al. 2017). High LNC values indicate a species has exploited the high nutrient environment; low LCC values indicate a species has not invested resources in longer-lived structures. Contradicting results in previous studies (Siebenkas et al. 2015), our results showed lower LNC in the fertilized plots. This could be due to the relatively low amount of fertilizer added to the plots. We also observed no phenotypic response in the LNC trait, at only 0.7%. Our results for LCC, however, were

congruent with previous studies (Liu et al. 2017), with values in unfertilized plots being higher than fertilized plots. LCC also had little phenotypic response, at only 9%; however, it followed in the direction of what would be expected for exploitative species.

While both multivariate and univariate analyses using CWTM values showed patterns of exploitative trait values being favored in fertilized plots, we found no relationship between effect size of abundance in response to fertilizer and the mean species trait values in fertilized plots. The discrepancy between analyses might be explained by the fact that the correlation analysis does not account for overall species abundance (either stem count or percent cover). Therefore, significant results in PERMANOVAs and ANOVAs may be strongly influenced by a few dominant species that show the expected pattern of exploitative trait values in fertilized plots.

We note also that community trait variation can be driven by traits not measured in this study. N-fixing species and perennials were often lost with fertilization (Suding et al. 2005), and clonal growth form can be a main driver of variation between fertilized and unfertilized plots (Gough et al. 2012). These traits were not considered in our study. Root traits and plant-microbe interactions may also play important roles in responses to fertilization (Cantarel et al. 2015).

In previous literature (Grassein et al. 2010), species with exploitative trait values for SLA, LNC, and LDMC were shown to have high intraspecific variation. This variation was attributed to both phenotypic response and genetic variation, which was determined by a common garden experiment. Phenotypic response in those functional traits (SLA, LNC, LDMC) can be maladaptive and costly. Species that exhibit plasticity often display lower fitness to “fixed” species when they display the same trait value (DeWitt et al. 1998). Therefore, it is expected that phenotypic response in the traits would be more common in exploitative species that excel in resource acquisition. In our experiment, however, a species’ ability to respond

phenotypically showed no association with its resource use strategy, as measured by trait values. Rank correlation analysis found no relationship between the magnitude of a species' phenotypic response and its mean trait value. Thus, species with exploitative trait values did not have higher phenotypic response.

Several studies (Nicotra et al. 2010, Grainger and Turkington 2013, Li et al. 2016) have suggested that a plant's ability to phenotypically respond to variation in environmental resources can be an indicator of its success in a range of environmental habitats (wet and dry, nutrient-limited and -unlimited). Others, however, have shown that phenotypic response is a weak, negative predictor of success in these habitats (Dostal et al. 2016). In a study of four boreal forest understory species, Grainger and Turkington (2013) showed that plasticity can be an important component to a species' resource strategy, but was not necessarily important in all dominant species' strategies. Nicotra et al. (2007) found that significant plasticity, in some traits, was adaptive for helping species colonize new areas. We predicted that species which had a high phenotypic response to fertilization would also have increased success in fertilized plots; however, our results found no significant relationship between species' phenotypic response and its effect size of abundance in response to fertilization. Species that had a higher magnitude of phenotypic plasticity were not more successful with nutrient addition.

We considered an alternative hypothesis that species with higher phenotypic response would be equally competitive in a variety of treatments. Species with a higher degree of phenotypic plasticity have been shown to occupy broader ecological niches (Richards et al. 2005); and species with the ability to match their environmental conditions through phenotypic response, should be able to be successful in a broader range of habitats. Therefore, effect size of abundance would not change. In a study of nine functional traits in 40 tree species, Cassia-Silva



et al. (2017) found species with the ability to respond phenotypically were more widely distributed across habitats. However, Mitchell et al. (2016), in a study of four functional traits, found little support for the hypothesis that plant species with higher trait variability would be able to occupy a broader range of wet-to-dry habitats, with only variability in SLA being an indicator of success. In our study of functional traits, we predicted, based on this hypothesis, that species with high phenotypic response would, overall, be equally abundant in fertilized or unfertilized plots (effect size near zero), whereas species with low response would be substantially more successful in either fertilized or unfertilized plots (effect size strongly positive or negative). We found no support for this hypothesis; inspection of a scatterplot of effect size of fertilizer on abundance vs. magnitude of phenotypic response across species did not show the expected pattern.

While there was a trend in phenotypic plasticity toward exploitative trait values with fertilization, the degree and even the direction varied among species. For example, significant trait shifts in SLA in response to fertilization were mostly positive, as in *Eupatorium rotundifolium*, which showed a 40% increase. In contrast, *Lespedeza capitata* showed a decrease of 13% in SLA (Table 3). We acknowledge that phenotypic response we measured in functional traits could be in response to environmental factors other than fertilization, especially in those species that were collected in fewer blocks. Furthermore, our study did not determine whether phenotypic response was adaptive. Whether opposing directions in plasticity in different species represents two different adaptive strategies is still unclear (Strand et al. 2004). We found that leaf area, height, and biomass increased with fertilization. Another open question is whether these changes in traits represents direct or indirect response to increased nutrient availability. In a direct response, plant size traits increase because nutrients limited growth. In contrast, an indirect

response could occur, for example, if plants responded to reduced light levels or stronger competition caused by increased biomass in fertilized plots (Borer et al. 2014). In previous studies, plants have been shown to react to competition with plasticity in functional traits (Burns and Strauss 2012).

Although we have considered the implications of differences in trait values in fertilized and unfertilized plots in the context of phenotypic plasticity, another possible explanation for these differences is genetic divergence. Environmental filtering may act on genotypes as it does on species, selecting for certain genotypes that thrive in a high nutrient environment. Rapid evolution, which is genetic adaptation that happens in an ecological time scale, has been shown to occur (Hairston et al. 2005), and what we have interpreted as phenotypic response in this study may be based on evolved genetic differences. Based on a survey of long-term ecological studies, Strauss et al. (2008) suggest that adaptation to ecological manipulations can happen in relatively short time spans. Solely phenotypic changes happen more rapidly than genetic adaptation, and the results of phenotypic response are more readily reversed. However, studies of functional traits have generally found that phenotypic plasticity in response to environmental gradients accounts for more variation than genetic differences. For example, in an experimental study of functional traits in different environmental conditions in two subalpine grass species, Grassein et al. (2010) found that 30 percent or less of the overall functional trait variation due to genetic differences within species.

Phenotypic plasticity seems a more likely explanation for trait shifts given the short time scale and small spatial scale of the study, but confirmation is needed. Moving forward, there are at least two ways to determine whether phenotypic differences are due to plasticity or genetic differences in response to fertilization. One possibility is a common garden study, which

involves taking individuals from varying habitats and moving them to a common site. If phenotypic variation is still present at the common site, the variation is likely due to genetic adaptation to fertilizer. Genetic marker studies can also be used to determine if populations are genetically distinct. Genetic differences in populations are particularly well-studied in invasive plant species, as invasive populations often have differences in trait expression or resource strategy compared to their native counterparts (Zou et al. 2007). Alternatively, a greenhouse study could be used to experimentally test for phenotypic plasticity using clonal ramets. Siebenkas et al. (2015) used a greenhouse study to determine if species were expressing phenotypic response. Such experiments can test for the presence of phenotypic response in a species; however, Strauss et al. (2008) suggests that phenotypic response may take time to develop.

Our study provides evidence that moisture gradients can cause a change species and functional trait composition. In multivariate analysis, species composition was strongly significantly different based on proximity to the ditch, and analysis of the long-term data shows a strongly significant effect as well (C. Goodwillie, unpublished data). Specifically, plots near to, and presumably drained by, the ditch contained fewer wetland specialist species. Multivariate analysis of CWTM values also found that functional trait composition differed significantly between plots near to and away from the ditch. Individual traits also varied based on proximity to the ditch; four traits were significantly different in relation to proximity to the ditch for both fixed-species and treatment-specific CWTM analyses. For most traits, the plant communities in plots near the ditch with drier soils were shifted in the direction of exploitative species, although in contrast, FH had lower mean trait values in the plots close to the ditch. Overall, our results showed that functional traits varied in response to a soil moisture gradient similar to the response

to the nutrient gradient. This is consistent with the expectation that wetland plant species, which are adapted to low-nutrient environments, fall on the conservative end of the resource use and acquisition spectrum. Our results are generally consistent with previous studies of functional traits along moisture gradients. In a long-term succession experiment in a nutrient-poor wetland, Suter and Edwards (2013) found that over a decade, distinct experimentally-created plant communities converged based on their functional traits, with the most abundant species having high values for LDMC and seed mass and low values for SLA, relative growth rate, and LNC. Cassia-Silva et al. (2017), in a study of 40 tree species that occur in rocky savannah and savannah woodland habitats, also found a response in traits to soil moisture and nutrient levels.

Our research demonstrates that understanding of functional traits can provide insights about the biological mechanisms behind changes in plant community composition due to anthropogenic factors, including the addition of nutrients and changes in historical soil moisture levels. These changes can impact communities by contributing to loss of diversity (Roem et al. 2002, Soons et al. 2017) and alter a community's ecosystem services (Loreau et al. 2001).

Humans are constantly altering natural environments, both directly and indirectly. An example of direct alteration would be the application of fertilizer for agricultural crops. This addition of nutrients has impacts on the fields used to grow crops, as well as surrounding areas through fertilizer run-off (an indirect alteration). Another example of indirect alteration of nutrient levels includes atmospheric nutrient deposition from industrial pollution. Both of these examples can alter historical nutrient levels in favor of more exploitative species by shifting resource competition (Alvarez-Yepiz et al. 2017). Humans also alter historical soil moisture levels constantly, including ditching and draining soils for use in housing development and for agricultural use. We documented changes in species and trait composition in response to

fertilization and changes in soil moisture levels, and understanding these changes can aid in understanding how plant communities will respond in the face of unprecedented anthropogenic effects on the environment (Phoenix et al. 2006). We also found that some plant species respond phenotypically to fertilization, and that phenotypic response was ecologically important in shaping the plant community.

## TABLES

Table 1: Complete list of species sampled in study, including species abbreviation, plant family, habit, and traits sampled. Groups of traits sampled include leaf traits (L), plant size traits (S), and leaf nutrient traits (N). Comments include information about where the trait data was collected: Outside the plots (O) or unmowed plots (U). Taxonomic names followed Weakley 2015.

Species Name	Abbreviation	Family	Plant Type	Traits Sampled	Comments
<i>Acer rubrum</i>	ACRU	Aceraceae	Tree	L,S,N	U
<i>Amelanchier canadensis</i>	AMCA4	Rosaceae	Shrub	L,N	U
<i>Andropogon virginicus</i>	ANVI2	Poaceae	Graminoid	L,S,N	
<i>Aristida virgata</i>	ARVI5	Poaceae	Graminoid	L,S,N	
<i>Arundinaria tecta</i>	ARAR7	Poaceae	Graminoid	L,S,N	
<i>Aronia arbutifolia</i>	ARTE4	Rosaceae	Shrub	L,S,N	U
<i>Carex glaucescens</i>	CAGL5	Cyperaceae	Graminoid	L,N	
<i>Chasmanthium laxum</i>	CHLA6	Poaceae	Graminoid	L,S,N	
<i>Clethra alnifolia</i>	CLAL3	Clethraceae	Shrub	L,S,N	U
<i>Cyrilla racemiflora</i>	CYRA	Cyrillaceae	Shrub	L,S,N	U
<i>Dichanthelium lucidum</i>	DILU6	Poaceae	Graminoid	L,S,N	
<i>Dichanthelium scabriusculum</i>	DISC2	Poaceae	Graminoid	L,N	U
<i>Dichanthelium scoparium</i>	DISC3	Poaceae	Graminoid	L,S,N	
<i>Eupatorium capillifolium</i>	EUCA5	Asteraceae	Forb	L,S	
<i>Eupatorium recurvans</i>	EURE3	Asteraceae	Forb	L,S,N	
<i>Eupatorium rotundifolium</i>	EURO4	Asteraceae	Forb	L,S,N	
<i>Eupatorium semiserratum</i>	EUSE	Asteraceae	Forb	L,S,N	
<i>Euthamia caroliniana</i>	EUCA26	Asteraceae	Forb	L,S	
<i>Gratiola pilosa</i>	GRPI	Scrophulariaceae	Forb	L,S	
<i>Ilex glabra</i>	ILGL	Aquifoliaceae	Shrub	L,N	U
<i>Juncus dichotomus</i>	JUDI	Juncaceae	Graminoid	L,S	
<i>Juncus effusus</i>	JUEF	Juncaceae	Graminoid	L,N	
<i>Lespedeza</i>	LECA8	Fabaceae	Forb	L,S,N	

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<i>capitata</i>					
<i>Lespedeza hirta</i>	LEHI2	Fabaceae	Forb	L,S,N	
<i>Liquidambar styraciflua</i>	LIST2	Hamamelidaceae	Tree	L,S,N	U
<i>Lobelia nuttallii</i>	LONU	Campanulaceae	Forb	L,S	
<i>Magnolia virginiana</i>	MAVI2	Magnoliaceae	Tree	L,S,N	
<i>Nyssa sylvatica</i>	NYSY	Cornaceae	Tree	L,S,N	U
<i>Packera tomentosa</i>	PATO4	Asteraceae	Forb	L,S,N	
<i>Polygala cruciata</i>	POCR	Polygalaceae	Forb	L	
<i>Pteridium aquilinum</i>	PTAQ	Dennstaedtiaceae	Forb	L,N	U
<i>Pycnanthemum flexuosum</i>	PYFL	Lamiaceae	Forb	L,S,N	
<i>Rhexia mariana</i>	RHMA	Melastomataceae	Forb	L,S,N	
<i>Rhexia virginica</i>	RHVI	Melastomataceae	Forb	L,S,N	
<i>Rhus copallinum</i>	RHCO	Anacardiaceae	Tree	L,S,N	
<i>Rhynchospora inexpansa</i>	RHIN4	Cyperaceae	Graminoid	L,S,N	
<i>Rubus argutus</i>	RUAR2	Rosaceae	Subshrub	L,S,N	
<i>Rubus hispidus</i>	RUHI	Rosaceae	Subshrub	L,S,N	
<i>Scirpus cyperinus</i>	SCCY	Cyperaceae	Graminoid	L,S,N	O
<i>Scleria minor</i>	SCMI4	Cyperaceae	Graminoid	L,S,N	
<i>Smilax glauca</i>	SMGL	Smilacaceae	Shrub	L,S,N	U
<i>Smilax rotundifolia</i>	SMRO	Smilacaceae	Shrub	L,S,N	U
<i>Solidago pinetorum</i>	SOPI	Asteraceae	Forb	L,S,N	
<i>Solidago rugosa</i>	SORU2	Asteraceae	Forb	L,S,N	
<i>Solidago stricta</i>	SOST	Asteraceae	Forb	L,S,N	
<i>Symplocos tinctoria</i>	SYTI	Symplocaceae	Tree	L,N	U

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Table 2: Results for PERMANOVA of species composition, community-weighted trait means (CWTM) using a fixed-species trait value (see text for details), and CWTM using treatment-specific trait values. Fertilizer and ditch were treated as fixed factors; block was treated as a random factor nested within ditch.

Type	Source	df	Mean Square	<i>F</i>	<i>P</i>
Species Composition	Fertilizer	1	6041.9	11.318	0.002
	Ditch	1	4221.3	5.182	0.026
	Block(Ditch)	6	814.6	1.5259	0.063
	Fertilizer*Ditch	1	2384.8	4.4673	0.012
Fixed-Species CWTM	Fertilizer	1	46.318	3.6642	0.036
	Ditch	1	83.464	6.2405	0.026
	Block(Ditch)	6	13.375	1.0581	0.444
	Fertilizer*Ditch	1	11.451	0.90593	0.44
Treatment-Specific CWTM	Fertilizer	1	288.87	30.013	0.002
	Ditch	1	61.749	5.9506	0.023
	Block(Ditch)	6	10.377	1.0781	0.422
	Fertilizer*Ditch	1	6.6858	0.69464	0.498



Table 3: Results for phenotypic response of species in leaf and plant size traits. Number of individuals sampled from fertilized ( $N_F$ ) and unfertilized ( $N_U$ ) plots for each of the species is given. Pdiff is calculated as (mean fertilized trait value – mean unfertilized trait value) / mean unfertilized trait value. LRR is calculated as  $\log(\text{fertilized trait value} / \text{unfertilized trait value})$ .

Species	Leaf Area			Specific Leaf Area			Leaf Dry Matter Content			Final Height			Final Biomass		
	$N_F/N_U$	Pdiff	LRR	$N_F/N_U$	Pdiff	LRR	$N_F/N_U$	Pdiff	LRR	$N_F/N_U$	Pdiff	LRR	$N_F/N_U$	Pdiff	LRR
ACRU	16/14	0.449	0.161	16/14	-0.067	-0.030	16/14	0.598	0.204	5/5	0.365	0.135	5/5	0.569	0.196
AMCA4	5/5	0.426	0.154	5/5	-0.160	-0.076	5/5	-0.034	-0.015						
ANVI2	16/16	0.577	0.198	16/16	0.016	0.007	16/16	0.019	0.008	5/4	0.134	0.055	5/4	1.472	0.393
ARVI5	16/16	-0.067	-0.030	16/16	-0.166	-0.079	16/16	-0.028	-0.012	5/3	0.330	0.124	5/3	0.928	0.285
ARAR7	16/15	0.255	0.099	16/15	0.140	0.057	16/15	-0.217	-0.106	5/5	0.351	0.131	5/5	0.253	0.098
ARTE4	16/16	0.724	0.237	16/16	0.111	0.046	16/16	0.010	0.004	5/5	0.751	0.243	5/5	1.693	0.430
CAGL5	4/10	-0.119	-0.055	4/10	-0.021	-0.009	4/10	0.200	0.079						
CHLA6	16/16	-0.092	-0.042	16/16	-0.127	-0.059	16/16	-0.058	-0.026	5/5	0.600	0.204	5/5	1.264	0.355
CLAL3	15/16	-0.044	-0.020	15/16	0.315	0.119	15/16	-0.129	-0.060	5/5	0.242	0.094	5/5	-0.106	-0.049
CYRA	16/15	-0.125	-0.058	16/15	0.005	0.002	16/15	-0.084	-0.038	5/5	0.187	0.074	5/5	-0.121	-0.056
DILU6	16/16	0.257	0.099	16/16	-0.011	-0.005	16/16	-0.001	0.000	5/5	0.108	0.044	5/5	0.314	0.119
DISC2	10/10	-0.216	-0.106	10/10	-0.359	-0.193	10/10	0.145	0.059						
DISC3	16/16	0.109	0.045	16/16	0.159	0.064	16/16	-0.021	-0.009	5/5	0.206	0.081	5/5	0.024	0.010
EURE3	12/12	0.497	0.175	12/12	0.229	0.090	12/12	-0.032	-0.014	5/5	0.658	0.220	5/5	1.110	0.324
EURO4	16/16	0.127	0.052	16/16	0.403	0.147	16/16	-0.117	-0.054	5/5	0.429	0.155	5/5	1.292	0.360
EUSE	10/5	0.329	0.124	10/5	0.137	0.056	10/5	-0.207	-0.101	5/5	0.462	0.165	5/5	0.744	0.242
EUCA26	16/16	0.069	0.029	16/16	0.014	0.006	16/16	0.018	0.008	5/5	0.447	0.161	5/5	0.519	0.182
GRPI										3/2	0.933	0.286	3/2	6.636	0.883
JUDI										1/3	0.064	0.027	1/3	-0.212	-0.103
ILGL	16/16	0.254	0.098	16/16	0.337	0.126	16/16	-0.070	-0.032						
LECA8	16/16	-0.002	-0.001	16/16	-0.133	-0.062	16/16	0.068	0.029	5/5	-0.001	0.000	5/5	-0.063	-0.028
LEHI2										4/4	0.068	0.029	4/4	0.276	0.106
LIST2	16/16	-0.032	-0.014	16/16	-0.162	-0.077	16/16	0.009	0.004	5/4	0.532	0.185	5/4	0.797	0.254
LONU	12/12	0.677	0.224	12/12	0.454	0.162	12/12	-0.256	-0.129	2/3	0.466	0.166	2/3	2.524	0.547
MAVI2	16/16	0.218	0.086	16/16	0.736	0.240	16/16	-0.231	-0.114	5/5	0.605	0.205	5/5	0.613	0.208
NYSY	16/15	-0.087	-0.039	16/15	0.156	0.063	16/15	-0.063	-0.028	5/5	0.246	0.096	5/5	-0.074	-0.033
PATO4	16/16	0.974	0.295	16/16	0.123	0.050	16/16	-0.181	-0.087	5/4	0.722	0.236	5/4	1.273	0.357
PYFL	16/16	0.297	0.113	16/16	0.379	0.139	16/16	-0.105	-0.048	5/5	0.538	0.187	5/5	2.224	0.508
RHMA	16/16	0.497	0.175	16/16	0.033	0.014	16/16	0.034	0.015	5/5	0.499	0.176	5/5	0.222	0.087
RHVI	11/11	0.500	0.176	11/11	0.059	0.025	11/11	0.089	0.037	5/5	0.557	0.192	5/5	0.763	0.246
RHCO	16/16	1.314	0.364	16/16	0.195	0.078	16/16	-0.245	-0.122	5/5	0.536	0.187	5/5	2.109	0.493
RHIN4	16/16	-0.220	-0.108	16/16	-0.429	-0.243	16/16	0.028	0.012	5/5	0.700	0.230	5/5	1.526	0.402
RUAR2	16/15	0.496	0.175	16/15	0.121	0.050	16/15	-0.124	-0.057	4/5	1.525	0.402	4/5	5.464	0.810
RUHI	10/10	0.914	0.282	10/10	0.269	0.103	10/10	-0.159	-0.075	5/5	0.833	0.263	5/5	1.054	0.313
SCCY	11/11	-0.306	-0.159	11/11	-0.390	-0.215	11/11	0.580	0.199	5/5	-0.006	-0.003	5/5	0.037	0.016

SCMI4	16/12	0.349	0.130	16/12	0.002	0.001	16/12	-0.096	-0.044	5/5	0.176	0.070	5/5	-0.235	-0.116
SMGL	16/15	0.145	0.059	16/15	0.252	0.098	16/15	-0.333	-0.176	5/5	0.275	0.106	5/5	-0.146	-0.068
SMRO	16/16	0.301	0.114	16/16	0.234	0.091	16/16	-0.141	-0.066	5/5	0.612	0.207	5/5	0.514	0.180
SOPI										5/1	0.269	0.104	5/1	2.388	0.530
SORU2	16/16	0.376	0.139	16/16	0.202	0.080	16/16	-0.182	-0.087	5/5	0.279	0.107	5/5	0.230	0.090
SOST	16/16	0.324	0.122	16/16	0.307	0.116	16/16	-0.159	-0.075	5/5	0.351	0.131	5/5	0.826	0.262
SYTI	10/10	0.062	0.026	10/10	0.187	0.075	10/10	-0.103	-0.047						

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Table 4: Results of ANOVA using the fixed-species trait value for CWTM for each trait. Fertilizer and ditch were treated as fixed factors; block was treated as a random factor nested within ditch.

<b>Trait</b>	<b>Source</b>	<b>df</b>	<b>Mean Square</b>	<b>F</b>	<b>P</b>
Leaf Area	Fertilizer	1	105.813	9.056	0.024
	Ditch	1	344.307	22.764	0.003
	Block(Ditch)	6	15.125	1.295	0.381
	Fertilizer*Ditch	1	8.149	0.697	0.436
	Fertilizer*Block(Ditch)	6	11.684	1.431	0.233
	Error	32	8.162		
Specific Leaf Area	Fertilizer	1	467.531	16.625	0.007
	Ditch	1	1408.062	8.757	0.025
	Block(Ditch)	6	160.799	5.718	0.026
	Fertilizer*Ditch	1	34.544	1.228	0.310
	Fertilizer*Block(Ditch)	6	28.123	0.346	0.907
	Error	32	81.230		
Leaf Dry Matter Content	Fertilizer	1	0.002	2.180	0.190
	Ditch	1	0.001	0.449	0.528
	Block(Ditch)	6	0.002	1.401	0.346
	Fertilizer*Ditch	1	0.002	1.652	0.246
	Fertilizer*Block(Ditch)	6	0.001	3.803	0.006
	Error	32	0.000		
Leaf Nitrogen Content	Fertilizer	1	0.291	8.486	0.027
	Ditch	1	1.307	9.195	0.023
	Block(Ditch)	6	0.142	4.140	0.054
	Fertilizer*Ditch	1	0.030	0.859	0.390
	Fertilizer*Block(Ditch)	6	0.034	0.883	0.519
	Error	32	0.039		
Leaf Carbon Content	Fertilizer	1	150.125	1.081	0.339
	Ditch	1	0.015	0.000	0.989
	Block(Ditch)	6	67.164	0.484	0.801
	Fertilizer*Ditch	1	25.268	0.182	0.685
	Fertilizer*Block(Ditch)	6	138.897	3.074	0.017
	Error	32	45.189		
Carbon-Nitrogen Ratio	Fertilizer	1	808.153	9.966	0.020
	Ditch	1	24.328	0.098	0.765
	Block(Ditch)	6	248.060	3.059	0.100
	Fertilizer*Ditch	1	55.448	0.684	0.440
	Fertilizer*Block(Ditch)	6	81.087	4.417	0.002
	Error	32	18.358		

Final Height	Fertilizer	1	324.438	18.045	0.005
	Ditch	1	346.778	8.878	0.025
	Block(Ditch)	6	39.059	2.172	0.184
	Fertilizer*Ditch	1	53.541	2.978	0.135
	Fertilizer*Block(Ditch)	6	17.979	0.599	0.729
	Error	32	29.992		
Final Biomass	Fertilizer	1	17.932	5.063	0.065
	Ditch	1	4.566	2.891	0.140
	Block(Ditch)	6	1.580	0.446	0.826
	Fertilizer*Ditch	1	0.051	0.014	0.908
	Fertilizer*Block(Ditch)	6	3.542	1.334	0.271
	Error	32	2.655		

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Table 5: Community-weighted trait means for plots near and away from ditch using both fixed-species and treatment-specific trait means for species. Means shown in bold are significantly different at the 0.05 level in ANOVA.

Trait	Fixed-Species CWTM		Treatment-Specific CWTM	
	Far	Near	Far	Near
LA (cm <sup>2</sup> )	<b>11.413</b>	<b>16.770</b>	<b>14.653</b>	<b>22.720</b>
SLA (cm <sup>2</sup> /g)	<b>115.883</b>	<b>126.716</b>	<b>123.753</b>	<b>134.464</b>
LDMC (g/g)	0.343	0.336	<b>0.352</b>	<b>0.331</b>
LNC (% wt)	<b>1.500</b>	<b>1.830</b>	<b>1.478</b>	<b>1.780</b>
LCC (% wt)	82.448	82.413	81.891	81.597
CNR (% wt)	48.726	50.150	49.835	50.284
FH (cm)	<b>76.206</b>	<b>70.830</b>	94.343	92.067
FB (g)	4.168	4.784	6.792	8.899

Table 6: Results of ANOVA using the treatment-specific trait value for CWTM for each trait. Fertilizer and ditch were treated as fixed factors; block was treated as a random factor nested within ditch.

Trait	Source	df	Mean Square	F	P
Leaf Area	Fertilizer	1	1774.174	44.296	0.001
	Ditch	1	780.921	17.007	0.006
	Block(Ditch)	6	45.918	1.146	0.436
	Fertilizer*Ditch	1	42.703	1.066	0.342
	Fertilizer*Block(Ditch)	6	40.053	1.671	0.160
	Error	32	23.972		
Specific Leaf Area	Fertilizer	1	1054.922	65.933	<0.001
	Ditch	1	1376.649	19.561	0.004
	Block(Ditch)	6	70.378	4.399	0.047
	Fertilizer*Ditch	1	39.670	2.479	0.166
	Fertilizer*Block(Ditch)	6	16.000	0.245	0.958
	Error	32	65.208		
Leaf Dry Matter Content	Fertilizer	1	0.001	5.913	0.051
	Ditch	1	0.005	18.104	0.005
	Block(Ditch)	6	0.000	1.503	0.317
	Fertilizer*Ditch	1	1.61E-5	0.081	0.785
	Fertilizer*Block(Ditch)	6	0.000	1.847	0.121
	Error	32	0.000		
Leaf Nitrogen Content	Fertilizer	1	0.085	3.258	0.121
	Ditch	1	1.091	8.539	0.027
	Block(Ditch)	6	0.128	4.911	0.037
	Fertilizer*Ditch	1	0.073	2.819	0.144
	Fertilizer*Block(Ditch)	6	0.026	0.848	0.543
	Error	32	0.031		
Leaf Carbon Content	Fertilizer	1	58.619	0.483	0.513
	Ditch	1	1.033	0.011	0.919
	Block(Ditch)	6	92.273	0.760	0.626
	Fertilizer*Ditch	1	11.569	0.095	0.768
	Fertilizer*Block(Ditch)	6	121.383	2.960	0.021
	Error	32	41.010		
Carbon-Nitrogen Ratio	Fertilizer	1	1054.662	22.266	0.003
	Ditch	1	2.425	0.012	0.917
	Block(Ditch)	6	203.281	4.292	0.050
	Fertilizer*Ditch	1	6.922	0.146	0.715
	Fertilizer*Block(Ditch)	6	47.366	1.606	0.178
	Error	32	29.495		

Final Height	Fertilizer	1	14002.85	642.265	<0.001
	Ditch	1	62.161	1.158	0.323
	Block(Ditch)	6	53.662	2.461	0.149
	Fertilizer*Ditch	1	327.848	15.037	0.008
	Fertilizer*Block(Ditch)	6	21.802	0.298	0.933
	Error	32	73.194		
Final Biomass	Fertilizer	1	760.596	55.001	<0.001
	Ditch	1	53.295	7.424	0.034
	Block(Ditch)	6	7.179	0.519	0.778
	Fertilizer*Ditch	1	24.381	1.763	0.233
	Fertilizer*Block(Ditch)	6	13.829	2.072	0.085
	Error	32	6.675		

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Table 7: Spearman’s rank correlation of effect size of response to fertilization in abundance to log response ratio of phenotypic response in each trait; effect size of response to fertilization in abundance to the mean fertilized trait value; and mean unfertilized trait value to log response ratio of phenotypic response for each trait. Spearman’s rho is given for each interaction with sample size in parentheses. Asterisks denote significance at the 0.05 (\*), 0.01 (\*\*), and 0.001 (\*\*\*) levels.

	LA	SLA	LDMC	LNC	LCC	CNR	FH	FB
Effect Size vs Phenotypic Response	-0.045 (38)	-0.167 (38)	0.173 (38)	-0.034 (36)	0.097 (36)	0.014 (36)	0.149 (38)	0.035 (38)
Effect Size vs Mean Fertilized Trait Value	0.162 (41)	-0.227 (41)	0.141 (41)	0.140 (37)	0.114 (37)	-0.127 (37)	0.330*	0.357*
Mean Unfertilized Trait Value vs Phenotypic Response	0.013 (39)	-0.270 (39)	0.084 (39)	-0.289 (37)	0.291 (36)	-0.370*	-0.548***	-0.117 (38)



Table 8: Spearman's rank correlation of mean fertilized trait values for all traits: leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen content (LNC), leaf carbon content (LCC), leaf carbon-nitrogen ratio (CNR), final height (FH) and final biomass (FB). Spearman's rho is given for each interaction with sample size in parentheses. Terms in bold are significant at the 0.05 level for individual tests; terms in bold with an asterisk are significant at the table-wise level after sequential Bonferroni procedure.

	SLA	LDMC	LNC	LCC	CNR	FH	FB
LA	0.157 (41)	-0.102 (41)	0.297 (37)	<b>0.382</b> (37)	-0.170 (37)	0.172 (37)	<b>0.480*</b> (37)
SLA		<b>-0.591*</b> (41)	<b>0.502*</b> (41)	-0.272 (37)	<b>-0.572*</b> (37)	<b>-0.377</b> (37)	-0.148 (37)
LDMC			<b>-0.581*</b> (37)	0.169 (37)	<b>0.662*</b> (37)	0.145 (37)	-0.072 (37)
LNC				0.055 (37)	<b>-0.933*</b> (37)	-0.025 (33)	0.261 (33)
LCC					0.203 (37)	<b>0.488*</b> (33)	<b>0.435</b> (33)
CNR						0.125 (33)	-0.154 (33)
FH							<b>0.664*</b> (40)

# FIGURES

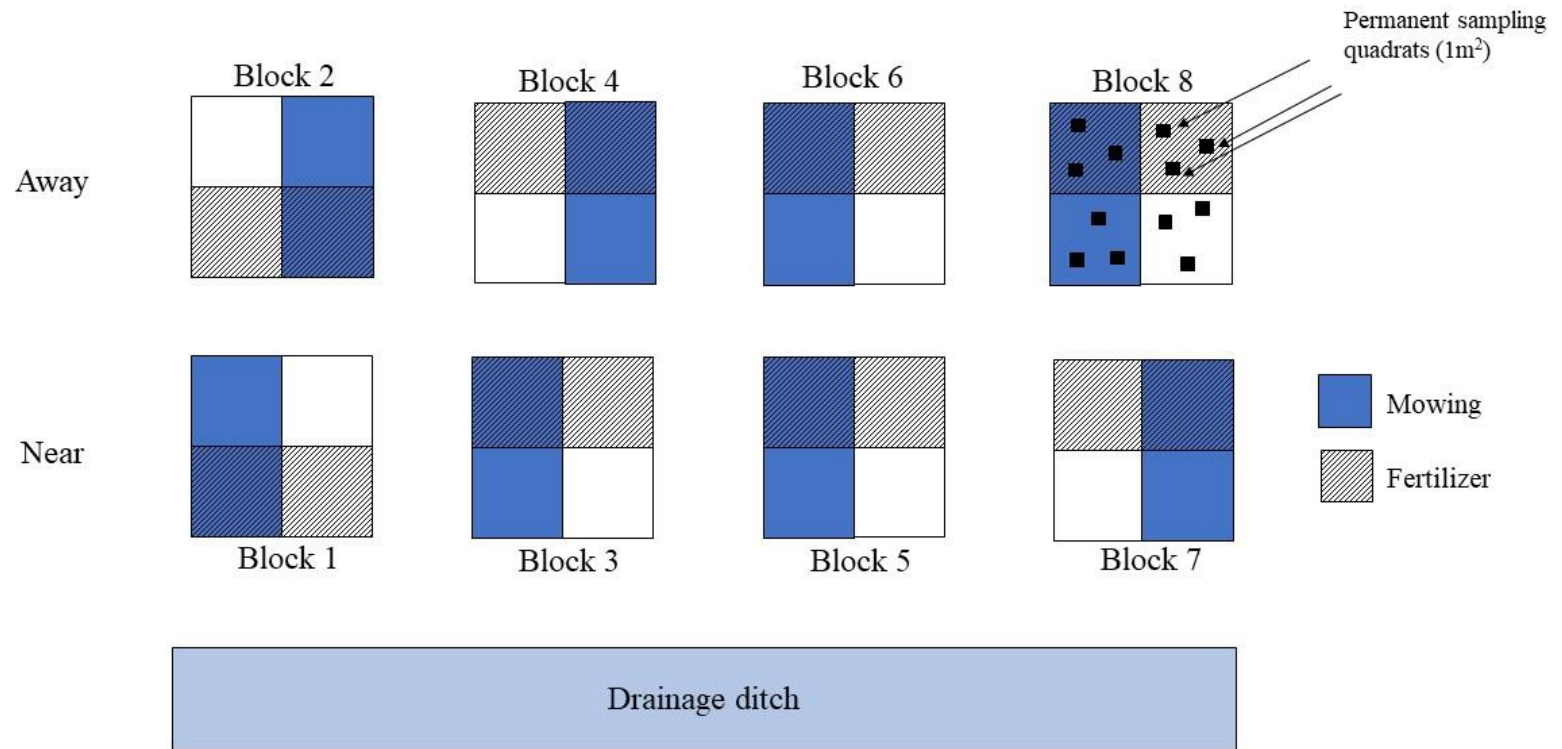


Figure 1: Experimental design of long-term study



Figure 2: Resource use strategy axis showing expected trait values for exploitative species.

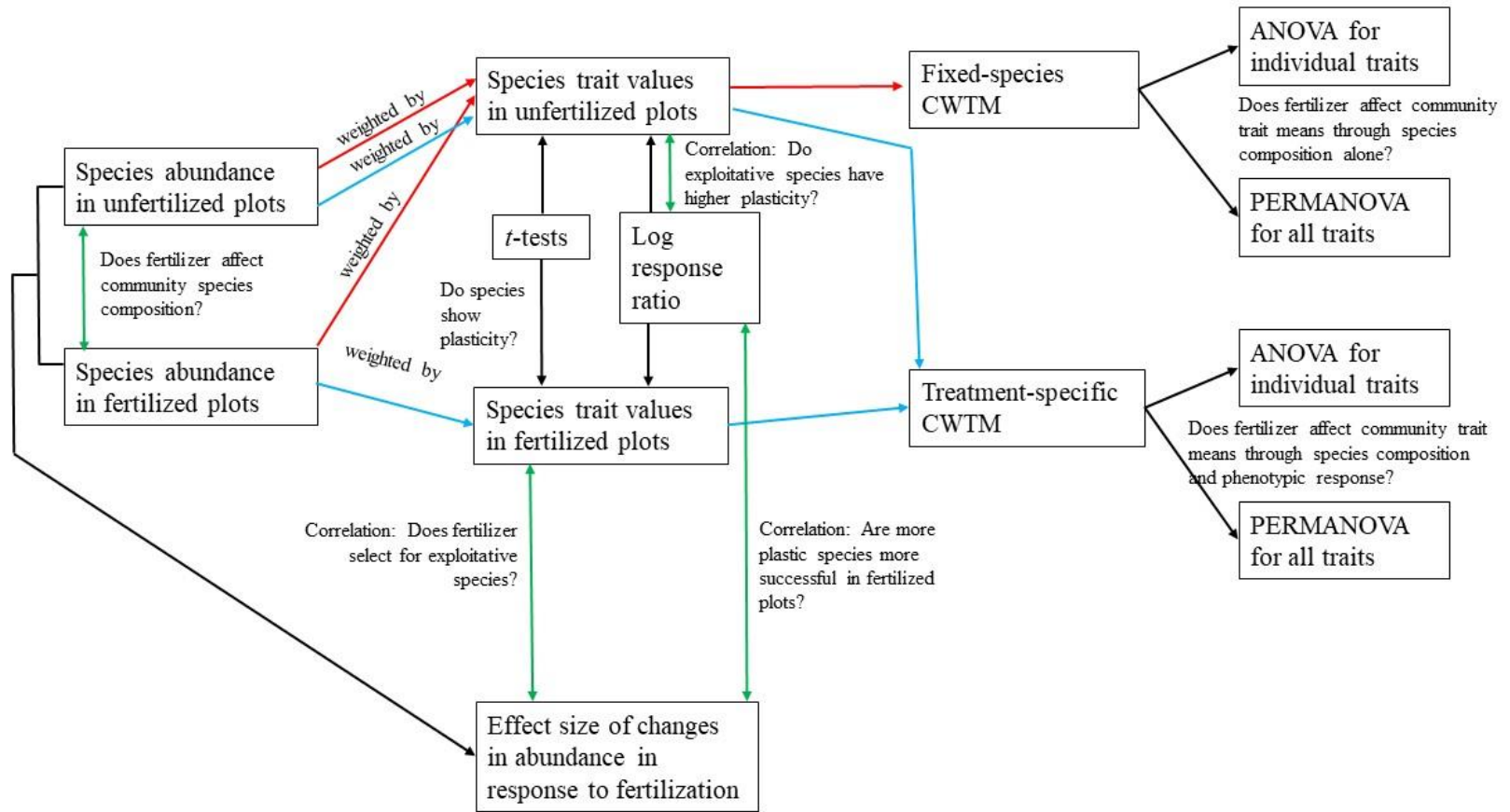


Figure 3: Diagram of all analyses and the questions they address. Red arrows represent calculation of fixed-species CWTM, while blue arrows represent calculation of treatment-specific CWTM. Green arrows represent a correlation analysis.

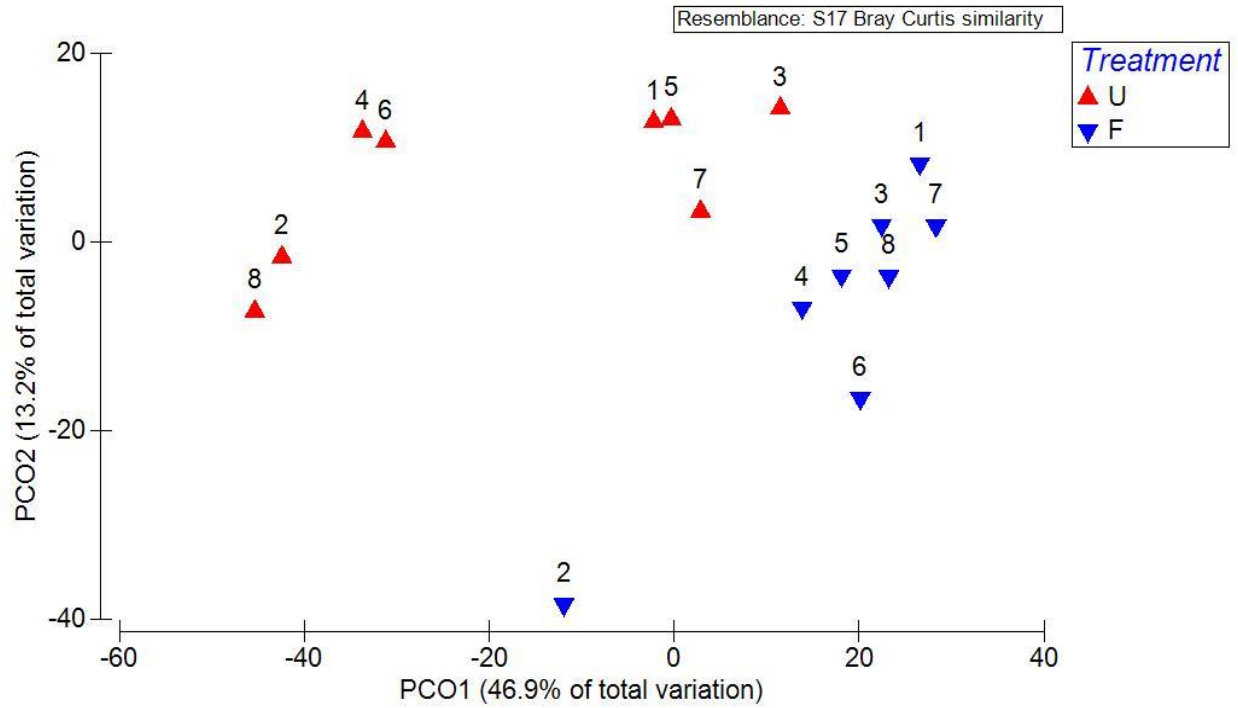


Figure 4: Principal coordinate analysis (PCO) plot of species composition in fertilized and unfertilized plots and plots near and away from the drainage ditch. Species abundance data (based on importance values) were averaged across three sampling quadrats per plot.

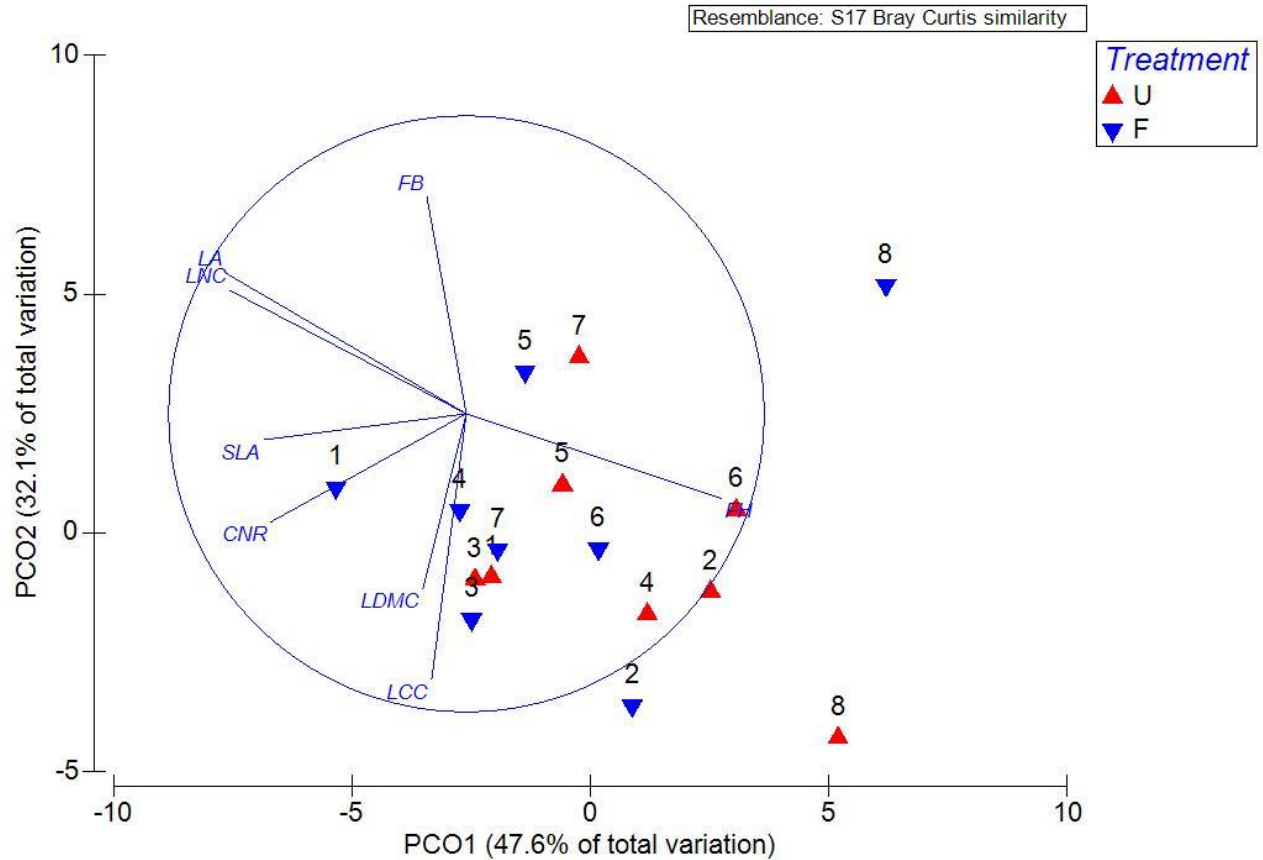


Figure 5: Principal coordinate analysis (PCO) plot of community-weighted trait means (CWTM) using fixed-species trait values in fertilized and unfertilized plots and plots near and away from the drainage ditch. Vectors denote the eight traits and their loadings: leaf area (Axis 1 loading: -0.8071, Axis 2 loading: 0.4715), specific leaf area (-0.6794, -0.0872), leaf dry matter content (-0.1460, -0.5876), leaf nitrogen content (-0.7941, 0.4140), leaf carbon content (-0.1164, -0.8929), carbon-nitrogen ratio (-0.6559, -0.3626), final height (0.8558, -0.2838), and final biomass (-0.1322, 0.7274). CWTM data were averaged across three sampling quadrats per plot.

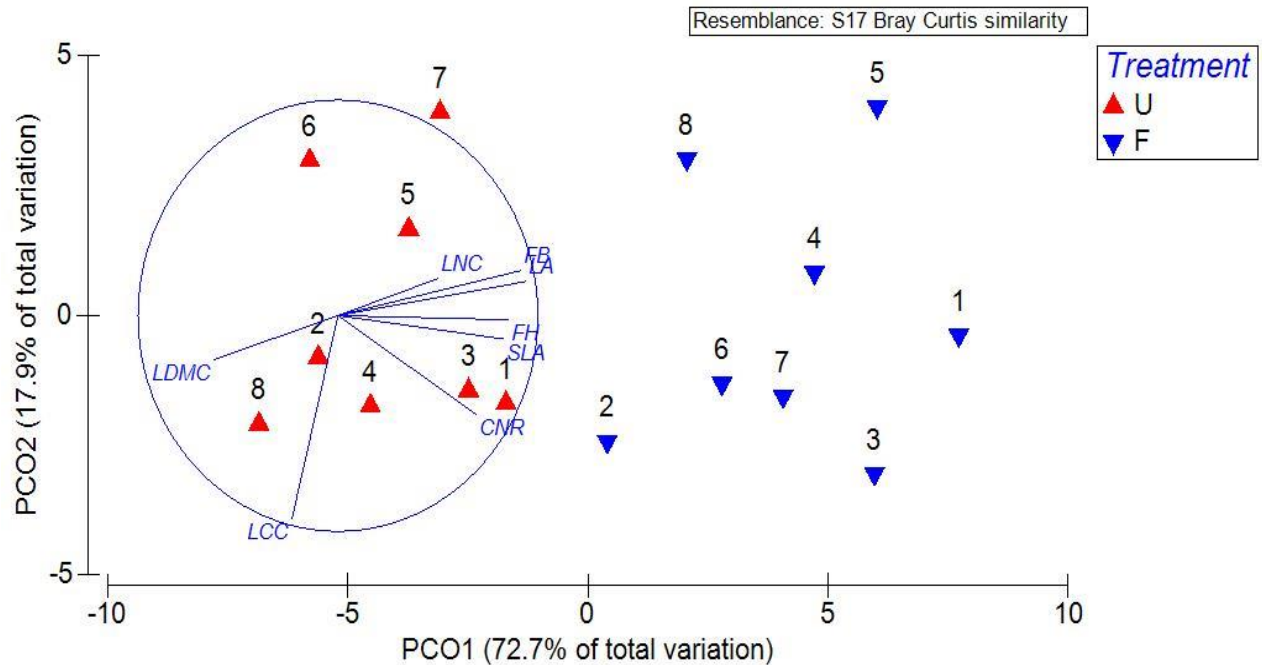


Figure 6: Principal coordinate analysis (PCO) plot of community-weighted trait means (CWTM) using treatment-specific trait values of fertilized and unfertilized plots and plots near and away from the drainage ditch. Vectors denote the eight traits and their loadings: leaf area (Axis 1 loading: 0.9390, Axis 2 loading: 0.1581), specific leaf area (0.8265, -0.1081), leaf dry matter content (-0.6183, -0.2034), leaf nitrogen content (0.5007, 0.1720), leaf carbon content (-0.2302, -0.9426), carbon-nitrogen ratio (0.6911, -0.4578), final height (0.8533, -0.0197), and final biomass (0.9129, 0.2083). CWTM data were averaged across three sampling quadrats per plot.

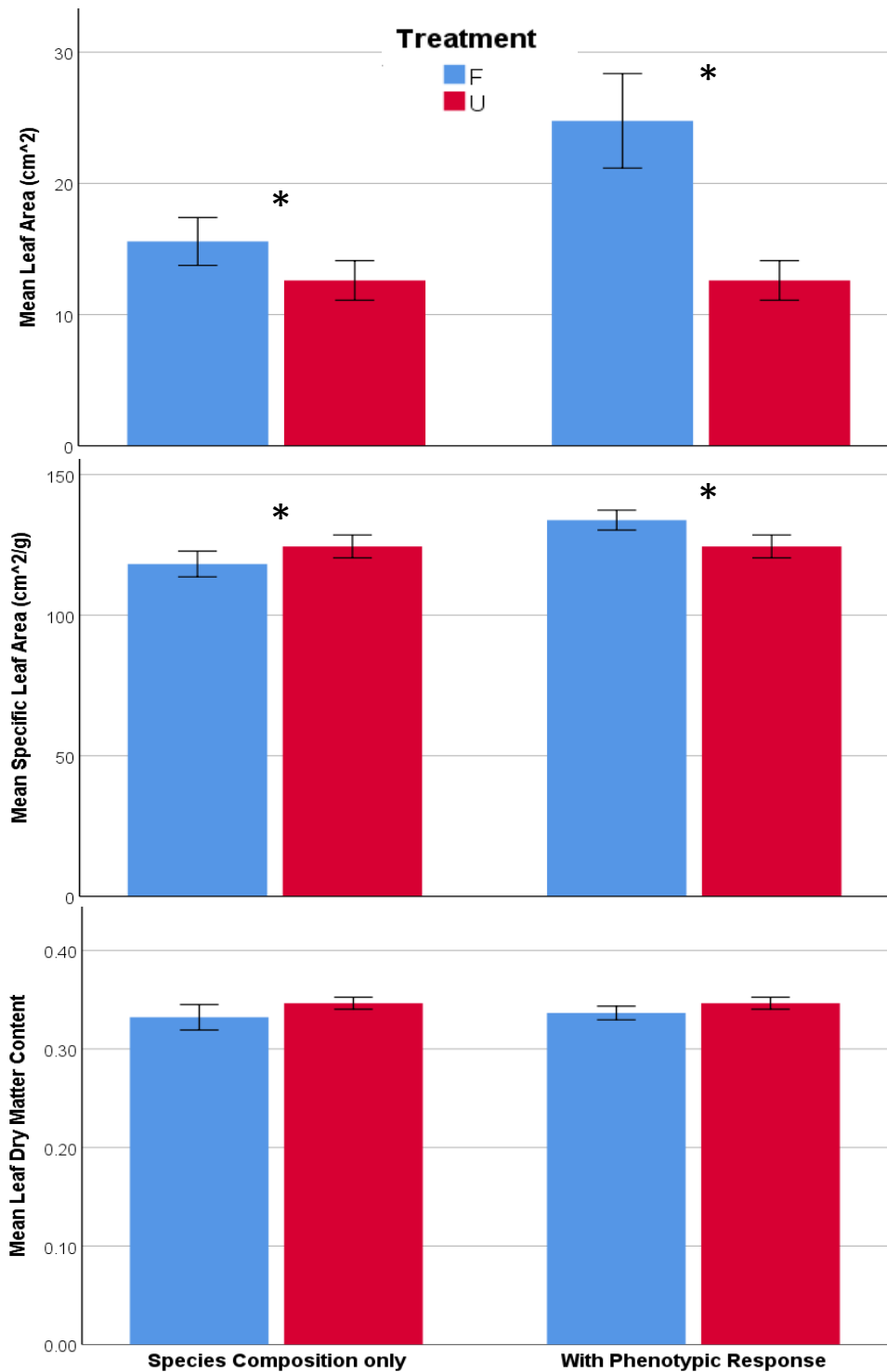


Figure 7: Community-weighted trait means (CWTM) in the individual leaf traits in fertilized and unfertilized plots. Fixed-species trait value CWTM are plotted in the left columns; treatment-specific CWTM plotted to the right. Asterisk denotes significance in ANOVA at the 0.05 level. Error bars denote the 95% confidence interval.



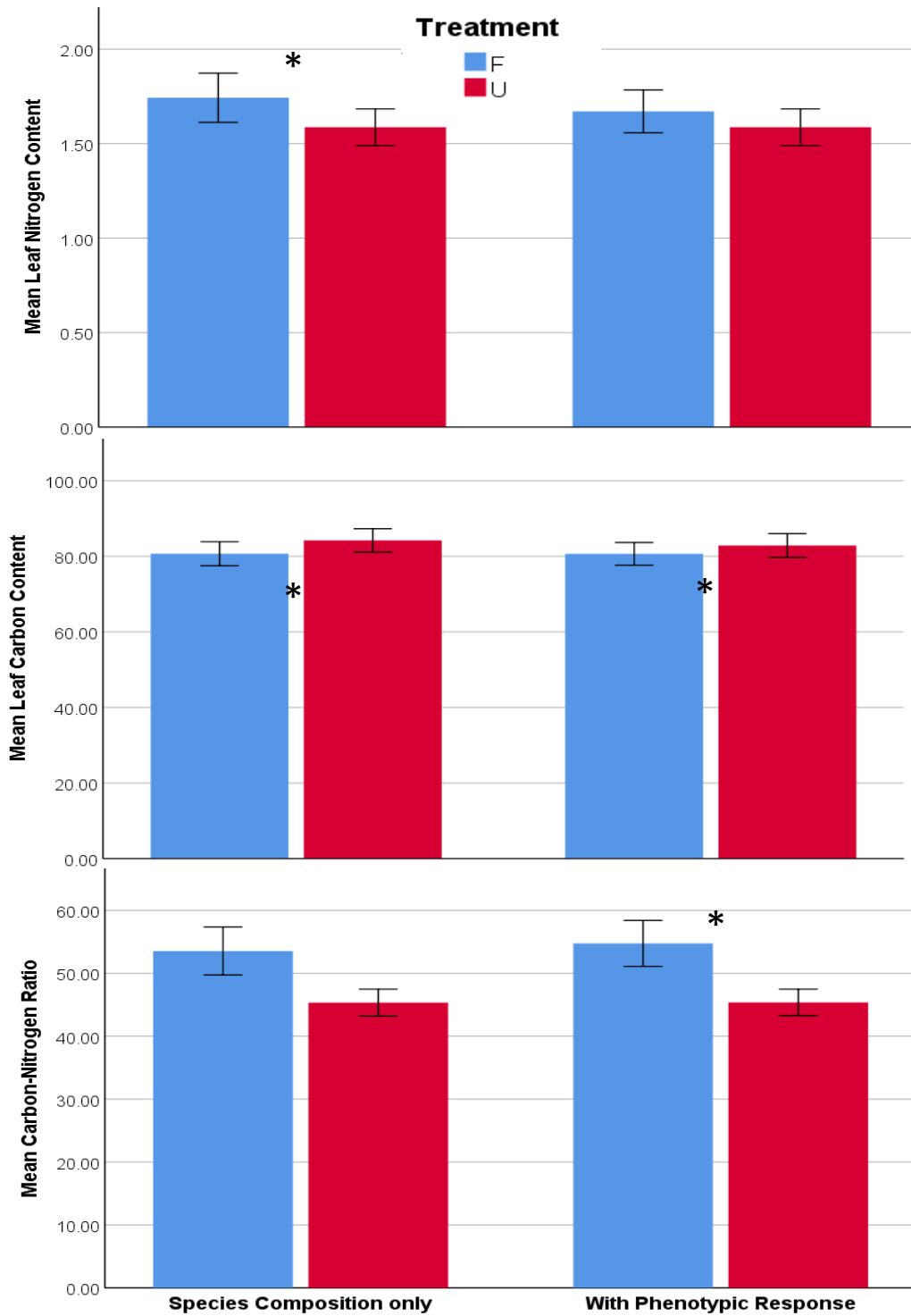


Figure 8: Community-weighted trait means (CWTM) in the individual leaf nutrient traits in fertilized and unfertilized plots. Fixed species trait value CWTM are plotted in the left columns; treatment-specific CWTM plotted to the right. Asterisk denotes significance in ANOVA at the 0.05 level. Error bars denote the 95% confidence interval.

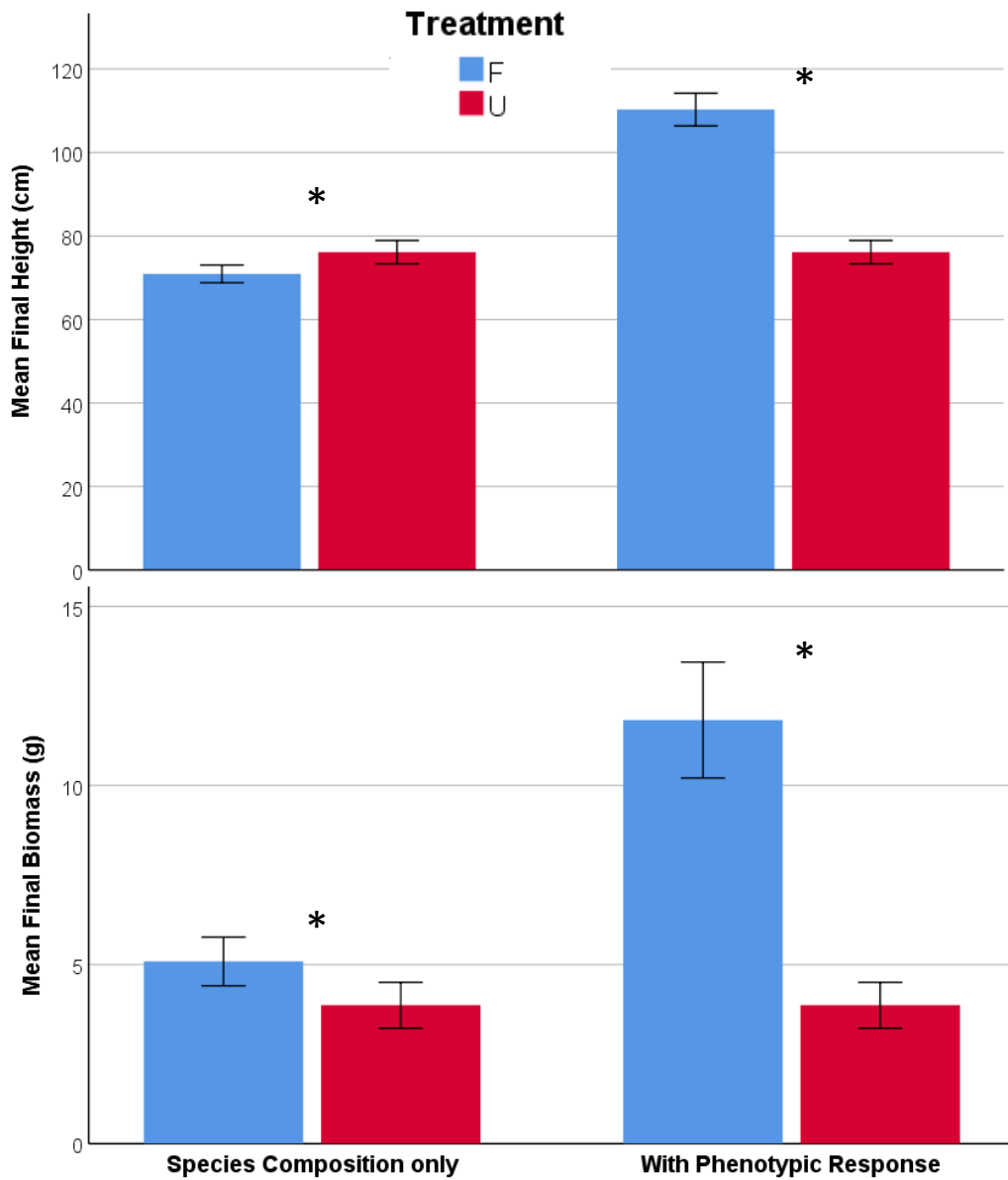


Figure 9: Community-weighted trait means (CWTM) in the individual plant size traits in fertilized and unfertilized plots. Fixed species trait value CWTM are plotted in the left columns; treatment-specific CWTM plotted to the right. Asterisk denotes significance in ANOVA at the 0.05 level. Error bars denote the 95% confidence interval.

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