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Factors affecting mycorrhizal colonization in Schizachyrium scoparium

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Factors affecting mycorrhizal colonization in *Schizachyrium scoparium*

by

Paul N. Frater

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Ecology and Evolutionary Biology

Program of Study Committee:
W. Stan Harpole, Major Professor
Brian Wilsey
Thomas Loynachan

Iowa State University
Ames, Iowa
2012
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DEDICATION

I would like to dedicate this thesis to my wife Haley without whose loving support I would never have been able to start or complete this work. I would also like to thank the graduate students of the Iowa State Department of Ecology, Evolution, and Organismal Biology for much support and friendship throughout my course of study.
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Last, but certainly not least, I would like to give a huge thanks to my wife Haley Frater. Without her I would have nothing.
Various factors have been known to influence the percentage of mycorrhizal colonization in plants. However, it is unknown whether certain factors matter at different scales or if one or several factors have an overarching effect on controlling rates of mycorrhizal colonization in plants. In this thesis I assessed the percentage of mycorrhizal colonization in *Schizachyrium scoparium* at 5 different sites that occur along a latitude gradient to determine which factors had an overarching effect and if there were site-specific differences in responses to nutrient addition. In the first chapter I present what factors affected the percentage of mycorrhizal colonization along a latitude gradient. I found that aridity was the only factor to contribute to changes in the percentage of mycorrhizal colonization. In my second chapter I used this information to account for differences between sites and assess responses in the percentage of mycorrhizal colonization among sites to see if there was a difference among sites. I found that the addition of nitrogen (N) decreased the percentage of mycorrhizal colonization in *Schizachyrium scoparium* across sites, but that the addition of phosphorus (P) had different effects among sites. Responses to the addition of P exhibited a U-shaped pattern in relation to ambient N:P ratio in soil at the sites. The results found in the first chapter of this thesis are novel and important to understanding plant-mycorrhizae relationships in plants while the results found in the second chapter agree with the current theory that exists to explain how resource stoichiometry affects the percentage of mycorrhizal colonization in plants.
Chapter 1. General Introduction

Introduction

Mycorrhizae are largely important for plant nutrient and water acquisition (Smith and Read 2008). While the existence of these organisms has been known for over 150 years (Nageli 1842) research into their importance has only been appreciated recently (Koide and Mosse 2004). Mycorrhizae assist plants in obtaining nitrogen (N), phosphorus (P), zinc (Zn), copper (Cu), and many other essential nutrients as well as water from the soil (Clark and Zeto 2000). In turn, plants provide the fungus with fixed carbon (C), which they obtain via photosynthesis (Bago et al. 2000). This symbiosis is dependent on a reciprocal transfer of plant C for nutrients from mycorrhizae (Fitter 1991, sensu Johnson 2009). However, rates of mycorrhizal colonization have been found to differ in plants growing in different environmental conditions (Johnson 1993, Treseder and Vitousek 2001, Johnson et al. 2003a) and the study of these differences is important to understanding the plant-mycorrhizae relationship. Recently, meta-analyses of these studies and accompanying theory have attributed differences in rates of mycorrhizal colonization in plants to resource conditions in the environment of the plant-mycorrhizae symbiosis (Treseder and Allen 2002, Johnson 2009). While the plant-mycorrhizae relationship has often been termed to lie somewhere along a gradient of mutualism to parasitism the idea of mycorrhizae parasitizing plants has not been well-tested and is perhaps ill-used. In contrast, studies of rates of mycorrhizal colonization have resulted in these rates changing with experimental manipulation to resource concentrations (Treseder 2004). This signifies that plants are able to alter rates of
mycorrhizal colonization in their roots (Graham and Eissenstat 1994) and do so in response to resource conditions. I was interested in knowing what factors affect mycorrhizal colonization in plants and if these factors differ across scales. Both soil nutrients and atmospheric carbon have been shown to influence aspects of mycorrhizal colonization in plants (Egerton-Warburton and Allen 2000, Rillig et al. 2000, Corkidi et al. 2002, Johnson et al. 2003a, 2003b, Chen et al. 2005). Nutrients tested for the most part have been N and P, and effects on mycorrhizal colonization of atmospheric C have been tested as well (Treseder 2004). These three resources have pretty clearly been shown to have an effect on rates of mycorrhizal colonization by generally decreasing percentages of mycorrhizal colonization with the addition of N and/or P and increasing these rates with the addition of atmospheric C. (Treseder 2004). These results are attributed to the trade balance and functional equilibrium of plants in exchanging C for nutrients with mycorrhizae (Treseder and Allen 2002, Johnson 2009). Plants should optimally allocate C to mycorrhizae in conditions where soil nutrients are low and mycorrhizae can help attain those resources or when there is an abundance of C produced by the plant. According to these principles increases in photosynthesis via increased temperature and growing season length should lead to increased C acquisition and in turn an increase in the percentage of mycorrhizal colonization in plants (Lambers et al. 1998, Johnson 2009). I wanted to know if changes in temperature and growing season length across a latitude gradient influenced the percentage of mycorrhizal colonization in Schizachyrium scoparium. I hypothesized that the percentage of mycorrhizal colonization would increase with decreasing latitude
because of the accompanying increase in mean annual temperature and growing season length. However, there are many environmental factors that change with latitude, so the quantification and inclusion in statistical analyses of factors thought to be important in altering rates of mycorrhizal colonization, other than nutrients, temperature, and light, is crucial in determining the importance of light and temperature on rates of mycorrhizal colonization in this study.

The first chapter of this thesis is a study that assessed rates of mycorrhizal colonization in *Schizachyrium scoparium* along a latitude gradient at sites that are part of a globally replicated nutrient alteration experiment. I collected root samples from five of these sites located in the central United States where *Schizachyrium scoparium* is most abundant and assessed the percentage of mycorrhizal colonization in these roots in order to determine the influences that nutrients, light, and temperature had, if any, on rates of mycorrhizal colonization in these plants. In order to account for other factors that may be important to rates of mycorrhizal colonization and that also change with latitude I have used a structural equation modeling approach that includes nutrient concentrations (soil N and P), temperature, growing season, pH, and aridity. I hypothesized that nutrients will have an effect on the percentage of mycorrhizal colonization in *Schizachyrium scoparium* as has been previously shown, but that mean annual temperature and growing season length at sites will also positively correlate to higher percentages of mycorrhizal colonization. That is, plants growing at sites with warmer temperatures and longer growing seasons will have increased rates of
mycorrhizal colonization.

Two major assumptions of this first chapter are 1) that the C budget of plants actually increases with increased temperature and growing season, and 2) that plants can control the relationship with mycorrhizae. The first assumption is stated with knowledge of plant photosynthetic physiology that rates of photosynthesis are known to increase with temperature (Lambers et al. 1998). It also follows that if plants photosynthesize for a greater number of days throughout the year that this will result in greater per annum rates of C acquisition. The second assumption has been shown to exist in experimental microcosms with more and less mutualistic mycorrhizae and plants being able to reduce C flow to less mutualistic mycorrhizae (Kiers et al. 2011), which would eventually decrease rates of these mycorrhizae. In addition, this assumption is invoked because plants are facultative on mycorrhizae whereas mycorrhizae are obligate biotrophs of plants.

The second chapter of this thesis presents a study that assessed responses in the percentage of mycorrhizal colonization to nutrients across sites. A variety of field studies have looked at rates of mycorrhizal colonization in response to nutrient addition (Treseder and Vitousek 2001, Johnson et al. 2003a, Treseder 2004); however, many of these studies fail to neglect inter-site differences that may affect mycorrhizal colonization. It is unknown if differences in precipitation, aridity, pH, and ambient nutrient conditions across sites play a role in determining percentages of mycorrhizal
colonization in plants. Mycorrhizae have been attributed to influencing water uptake (Smith and Read 2008) and water use efficiency (Hobbie and Colpaert 2004) in plants. Similarly, variable results of the effects of pH on mycorrhizal colonization have been found (Richards 1961, Theodorou and Bowen 1969, Wang et al. 1993). I was interested in assessing changes in the percentage of mycorrhizal colonization in *Schizachyrium scoparium* in response to nutrient treatment among sites while accounting for the environmental changes found in the first chapter of this thesis. This will provide an idea for the role that nutrients actually play in the plant-mycorrhizal relationship. My specific research question for this chapter is to see if differences in nutrient factors among sites (e.g. nutrient concentration and stoichiometry) influence the response of the percentage of mycorrhizal colonization in *Schizachyrium scoparium* to nutrient fertilization.

**Thesis Organization**

The following thesis is organized into two data chapters and a conclusion. The first chapter discusses my study of assessing the environmental factors controlling the percentage of mycorrhizal colonization across latitude. The second chapter is on my work assessing responses of mycorrhizal colonization to nutrient treatment and how these responses were determined by soil nutrient conditions. Both of these papers are displayed in a format such as the manuscripts I will submit to peer-reviewed journals.
References


Chapter 2. Extent of mycorrhizal colonization in *Schizachyrium scoparium* along a latitude gradient

**Abstract**

Resource stoichiometry has been proposed to influence rates of mycorrhizal colonization in plants. This work has looked at environmental ratios of carbon, nitrogen, and phosphorus (C:N:P); however, most of these studies have not looked at overarching limitations or environmental controls on the plant-mycorrhizae relationship (e.g. energetic influences such as temperature or light, water limitation, or pH). I present research that assesses rates of mycorrhizal colonization in *Schizachyrium scoparium* along a latitude gradient. I hypothesized that mycorrhizal colonization would increase with decreasing latitude due to increased mean annual temperature (MAT) and growing season length (GSL) at lower latitudes, which would allow plants to photosynthesize both at higher rates and for longer periods throughout the year thereby increasing carbon budget. Mycorrhizal colonization did increase with decreasing latitude; however, using structural equation modeling (SEM) I show here that aridity was the main driver for this pattern. MAT and GSL were not able to significantly explain any patterns in the data. Similarly, differences in nutrients (i.e. N and P) when accounting for other factors had no significant effect on the percentage of mycorrhizal colonization in *Schizachyrium scoparium*. 
Introduction

The effect of nutrients and nutrient stoichiometry (specifically carbon - C, nitrogen - N, and phosphorus - P) on rates of mycorrhizal colonization has been studied extensively for at least the past 20 years (Bååth and Spokes 1989, Johnson 1993, Rillig et al. 2000, Titus and Leps 2000, Valentine et al. 2001, Treseder and Allen 2002, Johnson et al. 2003, Treseder 2004, Johnson 2009). Stoichiometry of resources has been hypothesized to increase or decrease mycorrhizal colonization in plants due to changes in supply and demand in biological markets of plants and mycorrhizal fungi (Schwartz and Hoeksema 1998, Johnson 2009). However, research has for the most part only looked at the influences of soil nutrients and atmospheric carbon level on rates of mycorrhizal colonization in plants making this research purely stoichiometric in nature (Johnson 2009). Carbon clearly plays an important role in the plant-mycorrhizae relationship (Miller et al. 2002, Cameron et al. 2008), and much theoretical and recent empirical work has been done to establish how and when plants should cooperate with mycorrhizae and when they should abjure the relationship (Fitter 1991, 2006, Kiers et al. 2011). One would expect, all else being equal, that if the acquisition of carbon were to increase in plants, then the proportion of root system colonized by mycorrhizae would increase as well due to increased carbon allocation from plants. This prediction has been shown with experiments of increased atmospheric carbon (Rillig et al. 1999a, 1999b, 2000, Fransson et al. 2001, Lukac et al. 2003, Langley et al. 2003). However, no one has looked at how increased carbon from energetic influences (i.e. temperature, light) influences mycorrhizal colonization. Physiological principles of plant carbon
acquisition predict that photosynthetic rate increases with increased light and temperature, and indeed, increases in temperature and light have been shown to increase carbon acquisition in plants (Blackman and Matthaei 1905, Matthaei 1905). Therefore, an increase in carbon acquisition via increased photosynthesis should lead to higher rates of mycorrhizal colonization in plants. In addition to these factors mycorrhizal colonization has been shown to be important in the acquisition of water (Hardie and Leyton 1981, Mathur and Vyas 2000), and pH has been shown to have variable results on the percentage of mycorrhizal colonization in plants with the greatest rates of colonization seeming to occur at neutral pH values (Richards 1961, Theodorou and Bowen 1969, Wang et al. 1993, Bollag and Leneowicz 1984). Therefore, both water limitation and pH could also potentially play a large role in determining rates of mycorrhizal colonization in plants. I was interested in investigating which factors have the greatest effect on the percentage of mycorrhizal colonization in plants across a range of levels for these seemingly important factors.

To assess how these various factors affect rates of mycorrhizal colonization I collected root samples from Schizachyrium scoparium at five sites ranging in latitude from Minnesota to Texas. These sites range in mean annual temperature (MAT) from 7.0 - 18.9° C and in growing season length (GSL) from 139 - 235 days. This allows for a natural gradient of temperature and light that would be difficult to attain with a greenhouse study. This geographic gradient similarly contains a natural gradient of nutrient conditions, which allowed me to test for the effects of nutrient level and
stoichiometry on rates of mycorrhizal colonization as well. Additionally, the sites that I sampled are part of a replicated study of nutrient addition in grasslands, which extends beyond the natural range of nutrient conditions. This presents a unique opportunity to assess the status of this symbiosis with changes in nutrients as well as energetic components thereby offering a method in which to test the combined effects of the theory of ecological stoichiometry and energetic influences on rates of mycorrhizal colonization. Nutrient levels differ at these sites with P increasing and N decreasing with increasing latitude. While mycorrhizae are often considered more important when soil P concentrations are lower it has been shown that nutrient levels at particular sites determine how rates of mycorrhizal colonization respond to nutrient addition (Treseder and Vitousek 2001). This suggests that nutrient levels at a particular site are important in determining which nutrient (i.e. N or P) drive rates of mycorrhizal colonization at that site. Similarly, N:P ratio could be an explanatory variable alternative to strictly absolute values of N or P. A series of predictions can be made for how N, P, MAT, GSL, aridity (as a metric for water limitation), and pH affect rates of mycorrhizal colonization (Figs. 1-5). I hypothesized the following for each factor. Both higher N and P concentrations would decrease mycorrhizal colonization; however, I also expected N to be inversely correlated with latitude, but P to be positively correlated; I expected that P concentration would have a greater effect on mycorrhizal colonization since the bulk of literature has shown this (Treseder 2004), but that N may play a secondary role. N:P ratio, which I hypothesized to decrease with increasing latitude, should have a positive correlation with the percentage of mycorrhizal colonization. I predicted that both MAT
and GSL would increase the percentage of mycorrhizal colonization in plants, and would be inversely correlated with latitude. Aridity, I hypothesized, would have a positive correlation with both percentage of mycorrhizal colonization and an inverse correlation with latitude. I predicted that the percentage of mycorrhizal colonization would be affected by pH with a neutral pH having the greatest rates of mycorrhizal colonization; however, I did not have a good prediction at whether pH would increase or decrease along the latitude gradient sampled as I had not found any theoretical or empirical work to back up any prediction for this. Due to differences in inter-site N and P concentrations I expected both of these nutrients to have an effect on the percentage of mycorrhizal colonization thereby negating any latitudinal effect that the individual nutrients would have. Given the stoichiometric perspective of the relationship between plants and mycorrhizal fungi, and the importance of C:N:P ratios, I hypothesized that MAT and GSL would affect the percentage of mycorrhizal colonization the most. This prediction stems from theory that plants will photosynthesize at higher rates with increased MAT and GSL thereby increasing carbon for allocation to mycorrhizae, which should increase percentage of colonization.

Methods

Field Sites

I collected root samples of *Schizachyrium scoparium* at 5 replicated nutrient addition sites that are part of The Nutrient Network. The Nutrient Network is a global research cooperative that investigates top down and bottom up controls on grassland plant communities by employing nutrient and herbivory treatments to grassland sites around
the world (Stokstad 2011). Replicate treatments of N, P, and K with micronutrients were applied in a factorial block design to investigate singular and interactive effects of nutrients on various aspects of plant communities. All fertilizers added were pellet fertilizers that were added in the following quantities: N was added as time-release urea ($\text{N}_2\text{H}_4\text{CO}$) at a rate of 23 kg hectare$^{-1}$ year$^{-1}$. P was added as triple super phosphate ($\text{Ca(H}_2\text{PO}_4)_2$) at a rate of 51 kg hectare$^{-1}$ year$^{-1}$. K and micronutrients were fertilized together as a combination of potash (KCl) at a rate of 22 kg hectare$^{-1}$ year$^{-1}$ as well as 100 kg hectare$^{-1}$ year$^{-1}$ of Scotts Micromax, which is a blend of macro- and micro-nutrients (e.g. K, Ca, Mg, S, B, Cu, Fe, Mn, Mo, Zn). The 5 Nutrient Network sites where I collected roots from were: Cedar Creek LTER, MN; Chichaqua Bottoms, IA (CBGB); Barta Brothers Ranch, NE; Konza LTER, KS; and Temple, TX (which is located at the USDA-ARS Grassland Soil and Water Research Laboratory, Temple, TX). I chose these sites based on their involvement in the Nutrient Network and having replicate N, P, and K additions. All treatments were applied to 5 x 5 meter plots set up in a randomized block design. All sites had at least 3 replicate blocks with Cedar Creek LTER having 5 blocks and CBGB having 6 blocks. Sites were also chosen based on the prevalence of *Schizachyrium scoparium* at each site as well a location within the central regions of the species’ range. I chose this species because it is a dominant grass across much of the central United States, which makes findings widely applicable as well as allows me to test questions about ecological stoichiometry across a wide geographical range. Sites also contained additional herbivore exclosure treatments with one of these being unfertilized and another fully fertilized (i.e. with N, P, and K + micronutrients); however,
the exclusion of herbivores did not have a significant effect on the percentage of mycorrhizal colonization and was not included in analyses.

Field and Lab Methods

I collected root samples from *Schizachyrium scoparium* plants using a 1.75 cm diameter soil core to a depth of 30 cm. Samples were collected during the month of August, 2010. I collected samples by taking the root core just off the center of the bunchgrass to avoid destroying the meristem and harming the plant. Roots and soil samples were placed in sealable plastic bags and stored in a cooler with ice while in transport to the lab. They were then stored in a freezer at -20°C until processed. I retrieved roots from soil samples by sieving the samples through a 2mm soil sieve. I obtained fresh mass of roots, took a subsample between 0.05 g and 0.25 g for quantifying mycorrhizal colonization, and dried the rest of the root sample at 60°C for 48 hours in order to obtain a dry mass. The ratio between dry mass and fresh mass was used to calculate dried mass of the subsample used for assessing mycorrhizal colonization. Roots were stained in order to detect mycorrhizae using the trypan blue staining procedure modified from (Robertson et al. 1999). Modifications consisted of tailoring clearing and staining times to suit the roots of our study specimen as well as using filtered trypan blue solution instead of trypan blue powder. I then assessed the percentage of root sample colonized by mycorrhizal fungi using the grid-line intercept technique (Giovannetti and Mosse 1980).

Soil Analysis
I collected the following soil variables for each soil sample collected from the field: total C, total N, plant available P, pH, and texture. I was specifically interested in obtaining C, N, and P in order to calculate stoichiometric ratios of these. Total N was obtained instead of plant available N because of the time lag associated with transporting root and soil samples from the field sites to the lab. I was concerned that denitrification of inorganic N during transport would lead to an ill-represented sample of available N and therefore decided to use total soil N instead of plant available N.

Total C and N were obtained through the Soil and Plant Analysis Laboratory at Iowa State University using combustion analyses performed on a LECO CHN TruSpec and Elementar Variomax using the methods found in Pella (1990). I obtained available P by using the Mehlich III extract method and reading fluorescence at 630 nm on a BioTek Synergy HT multi-mode microplate reader. N:P ratio was calculated by dividing ppm of total N by ppm of available P. pH was measured by creating a slurry of 5 g of air dried soil with 10 ml of distilled water and shaking for 5 minutes, and then reading pH using a Fisher Scientific Accumet Basic AB15/15+ pH reader. Soil texture was taken from pre-treatment data collected at each site by the Nutrient Network.

**Environmental Variables**

I used 30 year climate normals from the NOAA National Climatic Data Center to obtain all climatic variables from the different sites (NOAA NCDC 2011). The climatic variables that I used were mean annual temperature (MAT), growing season length (GSL), and mean annual precipitation (MAP). I defined growing season length as the 90%
probability of the number of freeze free days above 0 degrees C (32° F). Soil information was obtained from the USDA Web Soil Survey (Web Soil Survey 2011). Aridity Index (AI) was used as a measure of water availability for plants. I calculated AI by the formula $AI = PET / P$, where $PET$ is potential evapotranspiration in mm and $P$ is total precipitation (in mm) (modified from UNEP 1997). Data on PET were obtained from EOS-WEBSTER by the University of New Hampshire (EOS-WEBSTER 2012).

**Statistical Analysis**

All statistical analyses were performed in R statistical computing software (R Development Core Team 2011). I used structural equation modeling (SEM) to parse out effects of the various factors thought to have an influence on mycorrhizal colonization. I also included latitude as having an effect on each of the factors in the model to control for the correlation that latitude had on each factor. I used the “lavaan” package in R to build models and assess model fit (Rosseel 2012) and used several model fit indices to assess the fit of the model including the Tucker-Lewis Index (TLI), Comparative Fit Index (CFI), chi-square, and root mean square error of approximation (RMSEA). I began by building an initial model that included all of the factors hypothesized above (Fig. 7), assessing fit of this full model using the above indices, and performing backwards selection until best-fitting model was achieved.
**Results**

**Variables Across Latitude**

The percentage of mycorrhizal colonization decreased significantly with increasing latitude (p < 0.001, $R^2=0.14$, Fig. 7). As expected, there were differences across latitude among the explanatory data variables that I tested (Fig. 8-14). Total N, log N:P ratio, pH, MAT, GSL, and aridity index (AI) all decreased with increasing latitude (N - p < 0.0001; $R^2=0.56$, log N:P ratio - p < 0.0001, $R^2=0.46$; pH - p < 0.0001, $R^2=0.68$; MAT - p < 0.0001, $R^2=0.99$; GSL - p < 0.0001; $R^2=0.88$; AI - p < 0.0001, $R^2=0.54$), while available P increased with increasing latitude (P - p < 0.0001, $R^2=0.18$).

**Variables Affecting the Percentage of Mycorrhizal Colonization**

The SEM with the best fitting model to the data included only pH, aridity index, and the percentage of mycorrhizal colonization as exogenous variables (chi-square = 154, d.f. = 1, p =0.21; TLI = 0.993; CFI = 0.999; RMSEA = 0.05; Fig. 16). Within this best-fitting model aridity index was the only variables that had a significant effect on the percentage of mycorrhizal colonization across latitude (p = 0.05, standardized coefficient=0.19). Since the original full model did not fit the data well this chosen model is considered an exploratory analysis, which is why I only report standardized coefficients for the model.

**Discussion**

I found that the percentage of mycorrhizal colonization in *Schizachyrium scoparium*
decreases with increasing latitude. To my knowledge, there is only one other study that looks at mycorrhizal fungi along a latitude gradient (Koske 1987), and this study looks more at dominance, richness, and community composition of mycorrhizae over ~3.5° of latitude. My study is novel in that it looks at rates of mycorrhizal colonization in plants over a range of latitude of ~15°. Rates of other mutualisms have been studied with regards to latitude, and it has been found that the number of mutualisms increase with decreasing latitude or are higher in tropics than in temperate zones (Schemske et al. 2009). This is exactly in line with what I have found; however, mechanisms for this pattern have not been established. I hypothesized that the increase in the percentage of mycorrhizal colonization moving towards southerly latitudes would be related to increased carbon acquisition from higher rates of photosynthesis due to higher MAT and a longer time span to photosynthesize throughout the year (increased GSL). However, aridity had a stronger influence on the percentage of mycorrhizal colonization. This does not necessarily mean that temperature and light do not have an influence on mycorrhizal colonization, but rather that aridity had a stronger influence in this study. It is both theoretically justified (Gillooly et al. 2001, Brown et al. 2004) and empirically shown that an increase in temperature will increase photosynthetic rate and similarly carbon assimilation in plants (Lambers et al. 1998). According to theory put forth by Johnson (2009) this should result in an increased percentage of mycorrhizal colonization. Future work on this subject should include studies that control for water and nutrient limitation, and assess rates of mycorrhizal colonization along light and/or temperature gradients.
I interpret the effects of aridity on the percentage of mycorrhizal colonization in terms of water limitation. Mycorrhizae have been shown to assist plants in the acquisition of water (Hardie and Leyton 1981, Mathur and Vyas 2000). If plants are limited by water (in terms of aridity), then it would make sense that this has an influence on the percentage of mycorrhizal colonization found in their root systems. The effect of water limitation on the percentage of mycorrhizal colonization could be tested by adding a water treatment to arid sites and assessing the change in the percentage of mycorrhizal colonization.

Aridity is important in terms of global change. As weather patterns continue to shift it may be possible to see larger rain events with longer dry spells in between (IPCC 2012). If this is the case plants may need to increase rates of mycorrhizal colonization in order to avoid water limitation during those spells. This would represent a large carbon cost for plants that previously would not have existed. Effects of this would lead to either increased carbon costs on the plant or increased water limitation, both of which could potentially have implications on populations of Schizachyrium scoparium and in turn community dynamics in prairies as this plant is pervasive and dominant throughout much of its range.
Fig. 1. Prediction of how the percentage of mycorrhizal colonization will change with increasing nitrogen or phosphorus levels in the soil.
Fig. 2. Prediction of how the percentage of mycorrhizal colonization will change with increasing N:P ratio in the soil.
Fig. 3. Prediction of how the percentage of mycorrhizal colonization will change with increasing mean annual temperature (MAT) and/or growing season length (GSL).
Fig. 4. Prediction of how the percentage of mycorrhizal colonization will change with levels of soil pH.
Fig. 5. Prediction of how the percentage of mycorrhizal colonization will change with increasing aridity.
Fig. 6. Predictions of how each of these factors that potentially have an impact on the percentage of mycorrhizal colonization in plants will change across latitude. Note that pH is predicted both ways as there is not currently knowledge of how pH will change across this latitude gradient.
Fig. 7. The relationship between the percentage of mycorrhizal colonization in *Schizachyrium scoparium* across latitude. This figure includes data points from all plots that were sampled including nutrient treatments. The pattern shown here is still significant when tested with control only plots as well (p < 0.05, $R^2=0.17$).
Fig. 8. Regression of total N across latitude; total N decreases with increasing latitude (p < 0.0001, $R^2=0.56$)
Fig. 9. Regression of available P across latitude; available P increases with increasing latitude (p < 0.0001, $R^2=0.18$)
Fig. 10. Regression of log N:P ratio across latitude; log N:P ratio decreases with increasing latitude (p < 0.0001, $R^2=0.46$)
Fig. 11. Regression of mean annual temperature (MAT) across latitude; MAT decreases with increasing latitude (p < 0.0001, R²=0.99)
Fig. 12. Regression of growing season length across latitude; growing season length decreases with increasing latitude ($p < 0.0001$, $R^2=0.88$)
Fig. 13. Regression of pH across latitude; pH decreases with increasing latitude (p < 0.0001, $R^2=0.68$)
Fig. 14. Regression of aridity index (AI) across latitude; AI decreases with increasing latitude ($p < 0.0001$, $R^2=0.54$)
Fig. 15 Full SEM model that I started with to explain which factors best explain the percentage of mycorrhizal colonization in *Schizachyrium scoparium*. 

MAT = Mean Ann. Temp.  
GSL = Growing Season Length
Fig. 16. SEM that has the best fit to the data (Chi-square=1.54, df=1, p=0.22; TLI=0.99; CFI=0.99; RMSEA=0.05). This model shows that only aridity had a significant effect on the percentage of mycorrhizal colonization in Schizachyrium scoparium across the latitude gradient sampled. Numbers reported are standardized coefficients.
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Chapter 3. Responses of the percentage of mycorrhizal colonization in *Schizachyrium scoparium* to nutrient additions and nutrient stoichiometry across 5 grassland sites

Abstract

Plant-mycorrhizae relationships have exhibited a range of rates of colonization under different resource conditions. Both parties may not exchange resources reciprocally based on environmental conditions. Resource stoichiometry has been invoked as a method for understanding the relationship between plants and fungi and how resource ratios alter proportions of mycorrhizal colonization presumably in order to maximize allocation of resources. However, inter-site differences are typically not assessed to evaluate if one nutrient has a greater impact on all mycorrhizal colonization in plants or if the driver for the relationship is site dependent. I used five sites of the Nutrient Network, a global grassland research cooperative, to assess the effects of nitrogen (N), phosphorus (P), and potassium (K) on mycorrhizal colonization of *Schizachyrium scoparium*, a dominant species throughout much of central North American. My objective for this study was to determine if the percentage of mycorrhizal colonization differs in response to nutrient treatment among sites, and if direction and magnitude of response is related to ambient soil nutrient conditions. Sites did differ significantly in the percentage of mycorrhizal colonization that was found in their root samples; however, with regards to site differences in response to nutrient treatment there was only a P x site interaction. Among all sites N addition decreased the percentage of mycorrhizal colonization in *Schizachyrium scoparium*. Ambient soil N:P ratio appeared
to have the greatest effect on the mean response of the percentage of mycorrhizal colonization with P treatment. The mean P responses were lowest at intermediate N:P ratios with low N:P ratios showing little response and high N:P ratios showing an increase in the percentage of mycorrhizal colonization. These results are evidence towards suggesting that local soil conditions at sites play a role in determining how plants will respond in the percentage of mycorrhizal colonization in their roots with nutrient addition.

**Introduction**

Mycorrhizae are important for nutrient acquisition in plants (e.g. nitrogen (N) and phosphorus (P)) (Clark and Zeto 2000, Smith and Read 2008); however, relationships between plants and mycorrhizae have been speculated to lie along a gradient of mutualism to parasitism depending on resource conditions (Johnson et al. 1997, Johnson 2009). There is a cost to the plant in the form of carbon (C) to maintain mycorrhizal relationships (Miller et al. 2002), and this cost is presumably not reciprocated equally by mycorrhizae when the nutrients they obtain are readily available (Fitter 1991). Nutrient limitation in plants creates a greater need for mycorrhizae to acquire nutrients than when those nutrients are at high levels (Treseder and Allen 2002). When nutrients are added to soil there is presumably no longer a reciprocal transfer of nutrients for C, and plants have been shown to decrease C allocation to mycorrhizae that do not equally reciprocate (Graham and Eissenstat 1994, Kiers et al. 2011, but see Grman 2012). This decrease in C allocation should result in a
reduction in the proportion of root system colonized by mycorrhizae.

Theory has been developed which uses ecological stoichiometry (or more specifically ratios of C:N:P, Sterner and Elser 2002) to explain the status of plant-mycorrhizae relationships under certain resource conditions (Treseder and Allen 2002, Johnson 2009). This theory predicts that when nutrients (i.e. N and P) are limiting to a plant then the plant should invest photosynthetic carbon to mycorrhizae in order to obtain these limiting soil nutrients. This idea of optimal allocation of photosynthetic carbon is in line with what Gleeson and Tilman (1992) predicted for plant allocation in relation to resource limitation (although this being with a symbiosis instead of plant organs), and is sometimes called “functional equilibrium” (Brouwer 1983). In this study I intend to assess the functional equilibrium of mycorrhizal colonization in the prairie grass *Schizachyrium scoparium* by assessing if and how different nutrients matter given ambient soil conditions. I take a plant-centric view of the plant-mycorrhizae relationship in this study for two reasons: 1) mycorrhizae are obligate fungi, whereas plants are facultative on mycorrhizae for growth and survival - this gives plants a benefit in this relationship because mycorrhizae need plants, but plants do not necessarily need mycorrhizae, and 2) plants have been shown to exhibit at least some control over the plant-mycorrhizae relationship (Kiers et al. 2011).

Studies that exhibit reductions of mycorrhizal colonization after nutrient addition have been shown empirically in a number of cases (Titus and Leps 2000, Dekkers and van
der Werff 2001, Treseder 2004). However, the differences in concentrations as well as stoichiometry of nutrients at various sites could have an effect on nutrient limitation at these sites thereby altering responses in mycorrhizal colonization to nutrient additions. Indeed, North American grassland sites have been shown to differ in their nutrient limitations (Craine and Jackson 2010).

By performing this study I intended to investigate how differences in site nutrient concentration and stoichiometry affected the response of the percentage of mycorrhizal colonization to added nutrients. I was interested in understanding how changes in the concentration and stoichiometry of soil nutrients (C,N,P) would impact mycorrhizal colonization in plants at various sites spanning a range of soil types and nutrient concentrations and ratios. I hypothesized that the addition of N will have a greater effect in reducing the percentage of mycorrhizal colonization in plants that are grown in soils low in N, and that P addition would lower mycorrhizal colonization at sites that have soils low in P. With regards to soil stoichiometry I hypothesized that as soil N:P ratios increased plants would show less of a response to N and more of a response to P addition. That is, at low N:P ratios plants would decrease the percentage of mycorrhizal colonization in their root system in response to N addition more than at high N:P ratios, whereas the opposite would be true for the response to P addition. This hypothesis stems from the idea that whichever resource is more limiting is the one that will have a greater effect on changes in the percentage of mycorrhizal colonization in plants.
Methods

Field Sites

I collected root samples of *Schizachyrium scoparium* at five replicated nutrient addition sites that are part of The Nutrient Network. The Nutrient Network is a global research cooperative that investigates top down and bottom up controls on grassland plant communities by employing nutrient and herbivory treatments to grassland sites around the world (Stokstad 2011). Replicate treatments of N, P, and K with micronutrients (K+) were applied in a factorial block design to investigate singular and interactive effects of nutrients on various aspects of plant communities. All fertilizers added were pellet fertilizers that were added in the following quantities: N was added as time-release urea (N₂H₄CO) at a rate of 23 kg hectare⁻¹ year⁻¹. P was added as triple super phosphate (Ca(H₂PO₄)₂) at a rate of 51 kg hectare⁻¹ year⁻¹. K+ was fertilized together as a combination of potash (KCl) at a rate of 22 kg hectare⁻¹ year⁻¹ as well as 100 kg hectare⁻¹ year⁻¹ of Scotts Micromax, which is a blend of macro- and micro-nutrients (e.g. K, Ca, Mg, S, B, Cu, Fe, Mn, Mo, Zn). All treatments were applied to 5 x 5 meter plots set up in a randomized block design. I collected root samples from the Nutrient Network plots at the following five sites: Cedar Creek LTER, MN; Chichaqua Bottoms, IA (CBGB); Barta Brothers Ranch, NE; Konza LTER, KS; and Temple, TX (which is located at the USDA-ARS Grassland Soil and Water Research Laboratory, Temple, TX; NutNet 2012). I chose these sites based on their involvement in the Nutrient Network and having replicate N, P, and K additions. Sites were also chosen based on the prevalence of *Schizachyrium scoparium* at each site as well as a location in the center of the range of the species. I
chose this study species because it is mycorrhizal and also because it is dominant across much of its range, which makes findings widely applicable as well as allowed me to test questions about ecological stoichiometry across a wide range. All sites had at least 3 replicate blocks with Cedar Creek LTER having 5 blocks and CBGB having 6 blocks. Sites also contained additional herbivore exclosure treatments with one of these being unfertilized and another fully fertilized (i.e. with N, P, and K+ micronutrients); however, the exclusion of herbivores did not have a significant effect on the percentage of mycorrhizal colonization in *Schizachyrium scoparium* in this study and so was not included in analyses.

*Field and Lab Methods*

I collected root samples from *Schizachyrium scoparium* plants using a 1.75 cm diameter soil core to a depth of 30 cm. Samples were collected during the month of August, 2010. I collected samples by taking the root core just off the center of the bunchgrass to avoid destroying the meristem and harming the plant. Roots and soil samples were placed in sealable plastic bags and stored in a cooler with ice while in transport to the lab. They were then stored in a freezer at -20°C until processed. I retrieved roots from soil samples by sieving the samples through a 2mm soil sieve. I obtained fresh mass of roots, took a subsample between 0.05 g and 0.25 g for quantifying mycorrhizal colonization, and dried the rest of the root sample at 60°C for 48 hours in order to obtain a dry mass. The ratio between dry mass and fresh mass was used to calculate dried mass of the subsample used for assessing mycorrhizal colonization. Roots were
stained in order to detect mycorrhizae using the trypan blue staining procedure modified from Robertson et al. (1999). Modifications consisted of tailoring clearing and staining times to suit the roots of the study species as well as using filtered trypan blue solution instead of trypan blue powder. Roots were cleared in a 2.5-5% potassium hydroxide solution at 90°C for 3-4 hours then left in the potassium hydroxide solution at room temperature for 6-8 hours. They were then treated in alkaline H₂O₂ for 1 hour, acidified in 1% HCl for 1 hour, and stained in a trypan blue solution made up of 1:1:1 (v:v:v) glycerol, lactic acid, water, and 1% (v) of 0.2 μm filtered trypan blue solution. The roots were stained in this solution at 90°C for 1 hour. Once stained, I assessed the percentage of mycorrhizal colonization in the root sample with the grid-line intercept technique (Giovannetti and Mosse 1980).

Soil Analysis

I collected the following soil variables for each soil sample collected from the field: total C, total N, inorganic P, pH, and texture. I was specifically interested in obtaining C, N, and P in order to calculate stoichiometric ratios of these. Total N was obtained instead of plant available N because of the time lag associated with transporting root and soil samples from field sites to the lab. I was concerned that denitrification of inorganic N during transport would lead to an ill-represented sample of available N and therefore decided to use total soil N instead of plant available N. Total C and N were obtained through the Soil and Plant Analysis Laboratory at Iowa State University using
combustion analyses performed on a LECO CHN TruSpec and Elemtr Variomax using the methods found in Pella (1990). I obtained inorganic P by using the Mehlich III extract method and reading fluorescence at 630 nm on a microplate reader. C:N and N:P ratios were calculated by dividing ppm of C by ppm total N and ppm total N by ppm available P, respectively. pH was measured by creating a slurry of 5 g of air dried soil with 10 ml of distilled water and shaking for 5 minutes, and then reading pH using a Fisher Scientific Accumet Basic AB15/15+ pH reader. Soil texture was taken from pre-treatment data collected at each site by the Nutrient Network.

Statistical Analyses

Statistical analyses were performed using R statistical computing software (R 2011). I used a linear mixed-effects ANOVA using the nlme package for R (Pinheiro et al. 2012), to test for differences in the percentage of mycorrhizal colonization in root samples collected among sites and fertilizer treatments. I treated block as a random variable to account for any differences among blocks. Aridity was not included as a random variable even though it was found to affect the percentage of mycorrhizal colonization because this should be adjusted for by using site as a fixed effect. To assess responses in the percentage of mycorrhizal colonization along ambient soil nutrient conditions I created a response metric of mycorrhizal colonization to nutrients for the treatments that had a significant site by treatment interaction. This response metric is the difference between the mean percentage of mycorrhizal colonization in treatment plots and the mean percentage in the control plots. I then fit regressions of this response
metric to mean ambient soil nutrient concentration and stoichiometric ratios for each site for factors that had a significant treatment by site interaction each.

**Results**

*Response to Nutrients Among Sites*

Sites differed significantly in the percentage of mycorrhizal colonization found in the roots of *Schizachyrium scoparium* (F=7.35, df=4,169, p < 0.0001). Among all sites the addition of N had a significant effect on the percentage of mycorrhizal colonization (F=8.10, df=1,169, p < 0.01). There was a significant P x site interaction (F=2.62, df=4,169, p < 0.05); however, there were no other nutrient by site interactions. This means that statistically speaking the percentage of mycorrhizal colonization in *Schizachyrium scoparium* does not respond differently to N or K+ among sites.

Significant variables can be seen in interaction plots showing the percentage of mycorrhizal colonization in control treatments and nutrient addition treatments for N and P (Fig. 17 and Fig. 18, respectively). Most sites decreased in mean percentage of mycorrhizal colonization in response to N (with the exception of Konza, which increased slightly but not enough to be significantly different). The response to P was more variable across sites, which is likely the reason why there was a significant P x site interaction.

*P Response to Ambient Soil Conditions*

Since P was the only factor to have a significant treatment by site interaction I assessed
the mean response in the percentage of mycorrhizal colonization in relation to ambient soil available P and N:P ratio. Ambient concentration of P in the soil did not have a significant effect on the mean response of the percentage of mycorrhizal colonization to P addition (p = 0.48, R² = 0.03); however, N:P ratio did have a significant U-shaped influence on the mean response of the percentage of mycorrhizal colonization to P addition (p < 0.05, R² =0.93, Fig. 19). At low N:P ratios mycorrhizal colonization did not decrease much, at intermediate N:P ratios the percentage of mycorrhizal colonization decreased the most, and at higher N:P ratios there was an increase in the percentage of mycorrhizal colonization with P addition.

**Discussion**

I was interested in determining if sites with different soil nutrient concentrations and stoichiometry responded differently to the addition of soil nutrients. I found that N addition decreased the percentage of mycorrhizal colonization overall, and that there was a significant P x site interaction (Table 4, Fig. 17). That both N and P make a difference to the percentage of mycorrhizal colonization is not surprising. Numerous studies have found that both N and P can decrease rates of mycorrhizal colonization in plants (Johnson 1993, Treseder 2004, Porras-Alfaro et al. 2007), but in certain instances the percentage of mycorrhizal colonization can increase with nutrient addition (Treseder and Allen 2002). This is thought to be due to direct soil nutrient limitation on fungus at low soil nutrient conditions, and a halt of carbon from plant to fungus at high nutrient conditions due to a decreased need for mycorrhizal fungi by the
plant. At intermediate soil nutrient concentrations conditions are thought to exist which allow for more reciprocal trade of carbon and nutrient between plant and fungus. Considering the model that Treseder and Allen (2002) present we would expect to see little response to nutrient addition at high nutrient concentrations because the percentage of mycorrhizal colonization at those soil concentrations would already be low. Alternatively, if soil conditions are presently low and nutrients are added, then an increase in the percentage of mycorrhizal colonization would be expected as it removes any possible nutrient limitation directly imposed on mycorrhizal fungi. Nutrients added to soil with ambient conditions conducive for a mutualistic trade of carbon for nutrients should drive the plant-fungus interaction into conditions where plants no longer need mycorrhizal fungi for nutrient acquisition and decrease carbon allocation to fungi thereby decreasing the percentage of mycorrhizal colonization. This type of pattern is what I found; however, it is for ratios of total N to available P in the soil. This suggests that nutrient limitation on plants and mycorrhizal fungi is not necessarily related to absolute soil nutrient concentrations, but rather the stoichiometric imbalance of resources in the soil.

N was a significant factor among all sites, and, for the most part, decreased the percentage of mycorrhizal colonization in *Schizachyrium scoparium* except at Konza Prairie where the percentage of mycorrhizal colonization remained relatively static with N addition (Fig. 17). It is interesting that the percentage of mycorrhizal colonization decreased with N addition overall. Although variable results have been
shown for N addition in other studies (Johnson et al. 2003) almost all sites in this study decreased in the percentage of mycorrhizal colonization with N addition except for one. Only two of these sites showed a significant response in the percentage of mycorrhizal colonization to N addition when assessed individually (Cedar Creek - ANOVA - F= 6.859, df=1.9, p < 0.05; CBGB - ANOVA - F=5.152, df=1.36, p < 0.05); however, these were the two sites that had five and six replicate blocks, respectively. It could be that a lack of statistical significance for N addition is related to fewer replicates (n=3) at the other sites, or it could be that the two sites that did show significant responses to N addition had lower soil N concentrations and lower N:P ratios. Similar results for responses to N have been achieved previously (Treseder and Vitousek 2001); however, opposite or null responses have also been shown (Corkidi et al. 2002). A decrease in the percentage of mycorrhizal colonization among all sites could also be related to nutrient limitation. Grassland sites have been shown to be primarily N-limited (Craine and Jackson 2010), and Konza, which is traditionally thought to be low in P, was the only site to not show any sort of response at all to N (Fig. 17). However, there was no significant effect of the mean response in percentage of mycorrhizal colonization to N addition when regressed against total soil N values or N:P ratio (N - p=0.36, R² =0.03; N:P - p=0.11, R² =0.49).

It seems that plants follow the functional equilibrium model with respect to mycorrhizal colonization and nutrient concentration and stoichiometry; however, this pattern is only evident when nutrient conditions are appropriate to allow mycorrhizae to seemingly avoid direct nutrient limitation. The fact that mycorrhizae are obligate
biotrophs on plants makes it difficult to test direct resource limitation on mycorrhizae since this limitation could ultimately be related to limits on plant photosynthesis and carbon production, and hence carbon allocation to mycorrhizae. My results seem to follow theory of nutrient concentration and stoichiometry on both mycorrhizal colonization in plants and direct nutrient limitation on mycorrhizal fungi; however, more work, including controlled greenhouse experiments, should be done to parse out the effects of nutrient status, stoichiometry, and nutrient limitation of mycorrhizae on plant-mycorrhizae relationships. An understanding of the controls of these variables on mycorrhizae and their relationships to plants are becoming increasingly important as nutrient deposition is a large component of global change. Knowledge of these relationships will give us insight into how they will be affected by such.
<table>
<thead>
<tr>
<th>Site</th>
<th>Soil Type</th>
<th>Soil Series</th>
<th>Soil Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar Creek LTER, MN</td>
<td>Sandy Outwash Plain</td>
<td>Zimmerman loamy fine sand and fine sand</td>
<td>Entisols</td>
</tr>
<tr>
<td>Barta Brothers Ranch, NE</td>
<td>Sandhills</td>
<td>Valentine fine sand</td>
<td>Entisols</td>
</tr>
<tr>
<td>Chichauqua Bottoms, IA</td>
<td>Sandy loam</td>
<td>Farrar fine sandy loam</td>
<td>Mollisols</td>
</tr>
<tr>
<td>Konza Prairie LTER, KS</td>
<td>Black clay soil</td>
<td>Temple, TX</td>
<td>Vertisol</td>
</tr>
<tr>
<td>Temple, TX</td>
<td>Black clay soil</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Soil texture for each site where samples were collected. Soil texture information was obtained from Nutrient Network pre-treatment data.

<table>
<thead>
<tr>
<th>Site</th>
<th>% Sand</th>
<th>% Silt</th>
<th>% Clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar Creek LTER, MN</td>
<td>90</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Barta Brothers Ranch, NE</td>
<td>96</td>
<td>3.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Chichauqua Bottoms Greenbelt, IA</td>
<td>88</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Konza Prairie LTER, KS</td>
<td>19</td>
<td>56</td>
<td>25</td>
</tr>
<tr>
<td>Temple, TX</td>
<td>31</td>
<td>31</td>
<td>38</td>
</tr>
</tbody>
</table>
Table 3. Total carbon, nitrogen, and available phosphorus for ambient (control plot) soils at sites where mycorrhizal samples were collected. Numbers reported in table are means, and numbers in parentheses are SE.

<table>
<thead>
<tr>
<th>Site</th>
<th>Total C (%)</th>
<th>Total N (%)</th>
<th>Available P (µg/g)</th>
<th>C:N</th>
<th>N:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cedar Creek LTER, MN</td>
<td>0.6 (0.06)</td>
<td>0.03 (0.004)</td>
<td>1.70 (0.13)</td>
<td>25.2 (4.0)</td>
<td>682 (95)</td>
</tr>
<tr>
<td>Barta Brothers Ranch, NE</td>
<td>0.4 (0.03)</td>
<td>0.03 (0.006)</td>
<td>0.13 (0.04)</td>
<td>16.3 (2.6)</td>
<td>17,066 (5731)</td>
</tr>
<tr>
<td>Chichauqua Bottoms Greenbelt, IA</td>
<td>0.5 (0.08)</td>
<td>0.05 (0.008)</td>
<td>1.55 (0.13)</td>
<td>19.2 (6.6)</td>
<td>1373 (265)</td>
</tr>
<tr>
<td>Konza Prairie LTER, KS</td>
<td>4.0 (0.2)</td>
<td>0.331 (0.017)</td>
<td>0.36 (0.03)</td>
<td>13.2 (0.12)</td>
<td>34,772 (2295)</td>
</tr>
<tr>
<td>Temple, TX</td>
<td>9.3 (0.1)</td>
<td>0.268 (0.017)</td>
<td>0.41 (0.05)</td>
<td>35.1(2.3)</td>
<td>27,690 (3567)</td>
</tr>
</tbody>
</table>
Table 4. Results from mixed-effects ANOVA treatment by site and all interactions on the percentage of mycorrhizal colonization in *Schizachyrium scoparium*. Significant factors are reported in bold.

<table>
<thead>
<tr>
<th>Factor</th>
<th>d.f.</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1 and 169</td>
<td>8.10</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>P</td>
<td>1 and 169</td>
<td>1.82</td>
<td>0.18</td>
</tr>
<tr>
<td>K</td>
<td>1 and 169</td>
<td>0.41</td>
<td>0.53</td>
</tr>
<tr>
<td>Site</td>
<td>4 and 169</td>
<td>7.35</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>N x P</td>
<td>1 and 169</td>
<td>0.34</td>
<td>0.56</td>
</tr>
<tr>
<td>N x K</td>
<td>1 and 169</td>
<td>1.46</td>
<td>0.23</td>
</tr>
<tr>
<td>P x K</td>
<td>1 and 169</td>
<td>2.08</td>
<td>0.15</td>
</tr>
<tr>
<td>N x Site</td>
<td>4 and 169</td>
<td>0.87</td>
<td>0.48</td>
</tr>
<tr>
<td>P x Site</td>
<td>4 and 169</td>
<td>2.62</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>K x Site</td>
<td>4 and 169</td>
<td>0.32</td>
<td>0.84</td>
</tr>
<tr>
<td>N x P x K</td>
<td>1 and 169</td>
<td>1.66</td>
<td>0.20</td>
</tr>
<tr>
<td>N x P x Site</td>
<td>4 and 169</td>
<td>0.69</td>
<td>0.60</td>
</tr>
<tr>
<td>N x K x Site</td>
<td>4 and 169</td>
<td>0.49</td>
<td>0.74</td>
</tr>
<tr>
<td>P x K x Site</td>
<td>4 and 169</td>
<td>0.45</td>
<td>0.77</td>
</tr>
<tr>
<td>N x P x K x Site</td>
<td>4 and 169</td>
<td>0.32</td>
<td>0.86</td>
</tr>
</tbody>
</table>
Fig. 17. Interaction plot showing the mean response to N addition across sites. Most sites decrease in the percentage of mycorrhizal colonization fairly consistently except for Konza, which shows an increase in the mean percentage of mycorrhizal colonization with N addition.
Fig. 18. Interaction plot showing the change in the mean percentage of mycorrhizal colonization with and without P addition. Sites show a wider range of responses to the addition of P, which is shown to be significant along ambient N:P ratios.
Fig. 19. The mean response of the percentage of mycorrhizal colonization to P addition per site plotted against mean ambient soil N:P ratios at respective sites. Response to P addition shows a significant U-shaped relationship along a range of N:P ratios ($p < 0.05$, $R^2 = 0.93$). Error bars shown are standard errors of the mean response on the percentage of mycorrhizal colonization to P treatment and ambient N:P ratios at sites. Note that the points used in this correlation are mean values per site as opposed to actual responses.
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Titus, J., and J. Leps. 2000. The response of arbuscular mycorrhizae to fertilization,


Chapter 4. Conclusion

This thesis detailed two components of factors that affect the percentage of mycorrhizal colonization in *Schizachyrium scoparium*. The first chapter looked at factors affecting the rate of mycorrhizal colonization across latitude, and the second chapter investigated how ambient soil conditions at sites affected responses in the percentage of mycorrhizal colonization to nutrient treatment.

In the first study I found that the percentage of mycorrhizal colonization in *Schizachyrium scoparium* decreased with increasing latitude. Using structural equation modeling I found this trend to be related to differences in aridity among sites.

In the second study, which focused on responses of mycorrhizal colonization rates to nutrient treatment, I found that N significantly decreased the percentage of mycorrhizal colonization in *Schizachyrium scoparium* and that there was a P x site interaction. The addition of N decreased the percentage of mycorrhizal colonization across sites while responses to the addition of P were site dependent. Depending on site the addition of P caused the percentage of mycorrhizal colonization to increase, decrease, or have no change at all. Upon further investigation I found both the direction and magnitude of response to P addition to be related to ambient N:P stoichiometry in the soil at a site. At sites with low N:P ratio there was little change in the percentage of mycorrhizal colonization. At sites with intermediate N:P ratio there was a decrease in the percentage of mycorrhizal colonization. While at sites with a high N:P ratio the addition...
of P actually increased the percentage of mycorrhizal colonization.

As previous research has shown there are a number of factors that affect rates of mycorrhizal colonization in plants. The majority of this prior research has focused on nutrient conditions in the soil (mostly N and P concentrations) and carbon dioxide addition. In the first chapter of this thesis I show that factors alternative to these (i.e. pH and aridity) are important in determining rates of mycorrhizal colonization in plants. Similarly, in the second chapter of this thesis I show that it is not simply N and P concentrations that are important in determining rates of mycorrhizal colonization, but also the stoichiometry of these resources. While I did not collect evidence to determine limitations to growth and optimal allocation of resources in this study, the findings could be interpreted as being related to such.

Across sites the overriding factor controlling the percentage of mycorrhizal colonization in *Schizachyrium scoparium* was aridity. I interpret this to mean that water limitation places a limit on plants and that plants respond by increasing mycorrhizal colonization to acquire water resources. This is also presumably why I did not detect an effect of nutrients across sites in the structural equation model. If aridity was the overarching factor affecting mycorrhizal colonization among sites, then nutrient input probably would not matter. Conversely, when we pick apart the data at a site-specific level and look at how nutrient additions affect the percentage of mycorrhizal colonization within sites there are results related to the ambient N:P ratio found in the
soil at sites. I interpret the results I have found and presented in chapter 3 of this thesis as stoichiometric-induced limitation placed on plants and mycorrhizal fungi, which differs among sites.

These results are important as a continuation for understanding the dynamics of the plant-mycorrhizae relationship. This relationship is incredibly complex as it can be affected by various environmental factors and strategy decisions by the participating organisms. In addition these influences can impact plants and mycorrhizal fungi independently, which can influence the relationship from multiple dimensions. Therefore, enhancing understanding of at least several aspects of this relationship is important. I have shown here that different factors can affect rates of mycorrhizal colonization in plants at multiple scales.

This study was also important in terms of revealing the factors affecting the percentage of mycorrhizal colonization in *Schizachyrium scoparium* across latitude. Aridity was important in determining rates of mycorrhizal colonization across latitude. More work is needed, including modeling and controlled greenhouse experiments, in order to fully understand how various factors affect rates of mycorrhizal colonization in this species. In addition, more theory needs to be developed to understand how changes in multiple factors such as those seen across latitude can affect plant-mycorrhizae relationships. My study only found that aridity influenced rates of mycorrhizal colonization; however, this does not mean that light and temperature have no effect on the relationship.
Current theory suggests that light and temperature should matter in this relationship. Modeling and controlled experiments with gradients of various factors such as water limitation should be performed in order to truly understand the complexity of factors affecting this relationship.

Understanding how these factors influence mycorrhizal colonization in this species will become increasingly important in light of global change. If aridity is changing due to increased drought conditions, then the effects of these could greatly influence rates of mycorrhizal colonization in *Schizachyrium scoparium*. It will be important to monitor this relationship and how it affects populations of this grass species because of its dominance across much of the central United States and prairie/plains ecosystems. Changes in the population of this plant could have drastic influences on these communities because of its dominance.