Evaluating relationships between plant traits and nitrogen use to help predict species' responses to climate change

A DISSERTATION

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loving grandfathers

Abstract

In many ecosystems, nitrogen (N) is the predominant nutrient limiting plant growth. Plants have therefore developed diverse strategies to compete for and partition soil N resources to ensure an adequate N supply. Differences in how plants acquire N may be important for predicting plant responses to different global changes. In particular, how species respond to climate change may depend on their N use strategy since climate change will likely alter the forms of N available to plants as well as total N availability. However, there remain key gaps in our understanding of plant N acquisition that impede our ability to project the impacts of climate change on plant communities. My research focuses on one of these gaps, the variation in plant use of different chemical forms of N, and examines how that variation can influence plant responses to climate change. Specifically, my research aims to increase our understanding of N acquisition in trees by examining whether plant traits can improve our ability to identify and explain differences in the use of different N forms. My first three chapters explore (1) the relationship between N uptake rates and root morphology for different N forms; (2) whether plant traits can help explain how species vary in their growth on different N forms; and (3) whether warming and drought alter patterns of N use in a regenerating forest. I then examine (4) how plant nutrient acquisition strategies and traits influence links between ecosystem carbon (C) and N cycling. Together, my research highlights that plants differ in their capacity to use different forms of N, which are in some cases associated with their traits. I also show that plants differ in how they partition N resources in the field, especially between mycorrhizal types. Finally, I show that both species' mycorrhizal type and phylogeny contribute to differences in C and N cycling in ecosystems where they

dominate. Overall, my research adds to our knowledge of how plants acquire N and shows that these strategies are an important influence on species and ecosystem responses to global change.

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INTRODUCTION

Forests cover almost one third of the earth's land surface (Bartholomé & Belward, 2005) and are a critical component of the global carbon (C) cycle (Del Grosso *et al.*, 2008). Like all ecosystems, the global distribution of forests will be impacted by climate change (Ma *et al.*, 2016), but the species within forest ecosystems may respond differently to these changes. Determining how the composition of forests will change is important as species can have distinct impacts on ecosystem processes (Hobbie *et al.*, 2007; Cornwell *et al.*, 2008a). An additional complicating factor is that while climate change can impact species directly, other factors change in tandem with climate that could impact how forest species respond. In particular, nitrogen (N) availability will likely change with climate (Rennenberg *et al.*, 2009; Melillo *et al.*, 2011; Bai *et al.*, 2013), so differences in how species acquire N could play an important role in structuring future plant communities and in turn influence ecosystem processes. Nitrogen is the main limiting resource in many northern forests (Vadeboncoeur, 2010; Crowley *et al.*, 2012), yet there are many aspects of plant N acquisition that are not well understood, which impedes our ability to predict plant responses to changes in climate.

This is especially true of differences between species in their use of different soil N forms (e.g. dissolved organic N, ammonium, and nitrate). Species differences in the use of each N form could be important because climate change will likely alter the availability of these forms (Rennenberg *et al.*, 2009; Bai *et al.*, 2013) and they are not necessarily equivalent N sources for plants. Each N form has distinct transporters for uptake and costs of assimilation (Miller & Cramer, 2004; Franklin *et al.*, 2017), and

species can differ in how quickly they take up different N forms as well as how well they grow on those same forms (McKane *et al.*, 2002; Hangs *et al.*, 2003; Boczulak *et al.*, 2014; Bloom, 2015b). However, it remains challenging to predict how species vary in their uptake rates of and growth on different N forms due to the variety of contexts in which it has been studied (Lucash *et al.*, 2007) and the number of factors that influence N uptake (Miller & Cramer, 2004; Britto & Kronzucker, 2013). Additionally, many studies have not examined how the combination of warming and changes in precipitation could alter N acquisition, which further impedes our ability to predict the consequences of climate change. Therefore, the overarching goal of my dissertation research was to evaluate whether using plant traits could help improve our understanding of how species differ in their use of different N forms and if the same traits impact how their use of N responds to changes in climate.

My dissertation explores several different plant traits and evaluates their usefulness in understanding species differences in N use in the context of climate change. Additionally, I explore the potential implications of changes in species composition on ecosystem processes in relation to similar traits. I do this by addressing the following questions:

1. How do N uptake rates vary with root morphological traits, and do these relationships vary by species and N form (*Chapter 1*)?
2. Can plant traits help explain variation in preferences for different N forms across species (*Chapter 2*)?

3. How do warming and rainfall reduction influence N uptake in a regenerating forest (*Chapter 3*)?
4. How do plant functional groups influence C and N cycling (*Chapter 4*)?

Together, these questions explore multiple aspects of plant N use to gain a better understanding of how different N forms could play a role in the response of plant species to climate change. I address these questions specifically in the context of the temperate forest – boreal forest ecotone, as this region contains species that vary in many plant traits related to N acquisition and changes in species composition may be seen earlier at the edges of species' ranges. The results reported broaden our understanding of the factors that influence plant N acquisition and how plants with different traits use N in both controlled and field conditions.

CHAPTER 1

Root morphological traits predict inorganic and organic N uptake rates in temperate and boreal forest tree species

Summary

Acquisition of nitrogen is critical for plant success, and nitrogen (N) uptake rates are frequently measured to compare species' abilities to compete for or partition N resources. However, most studies that compare N uptake rates across species ignore the potential influence of morphological traits on N uptake, even though morphology influences root surface length and area and thus the soil volume that roots are able to explore for a given mass investment. The length to mass ratio of a root is also indicative of physiological investment. Here we examine (1) how four root morphological traits (specific root length (SRL), root tissue density (RTD), diameter, root dry matter content (RDMC)) are related to N uptake rates across tree species, and (2) if these relationships differ across species and N forms. We hypothesized that N uptake rates would show positive (SRL) or negative (RTD, diameter, RDMC) relationships with root traits because for a given mass, longer, thinner roots have higher surface area for uptake. We expected the relationship to vary by species because of differences in which root order (related to root morphology) is absorptive and the kinetics of root N transporters. We also expected the relationship to vary by N form because of energetic differences in uptake and assimilation among N forms. To test our hypotheses, we measured root morphological traits and N uptake rates by depletion using excised fine roots from seven tree species. All traits were significant predictors of N uptake rates, and the direction of those

relationships matched our hypotheses, but SRL was the best predictor among the traits analyzed. Additionally, the majority of species had similar relationships between SRL and N uptake rate, though this relationship did vary by N form. Uptake rates were the highest for either NH_4^+ or NH_4^+ and glycine for all species, and NO_3^- uptake rates were low across all species. We expect that accounting for root morphology can improve comparisons of N uptake rates across species, and that plant N acquisition strategies combine both physiology and morphology to balance C investment in N uptake.

Introduction

Because nitrogen (N) is the predominant limiting resource in many northern forests, acquisition of N is critical to plant competitive success in these ecosystems (Högberg *et al.*, 2017). Species with higher N uptake rates may have greater capacity to acquire N from the soil than species with lower N uptake rates (McKane *et al.*, 2002; Hangs *et al.*, 2003); thus, N uptake rates have been considered as proxies for competitive success among species (Paulding *et al.*, 2010; Simon *et al.*, 2014). However, species can avoid competition by several strategies that allow them to partition N resources. Species can partition N acquisition by chemical form, space and/or time. For example, some species may "prefer" inorganic forms of N available in soil (such as nitrate, NO_3^-, and ammonium, NH_4^+) relative to organic forms (such as amino acids), exhibiting greater uptake rates for those N forms, whereas others show preference for the organic forms of N (McKane *et al.*, 2002; Simon *et al.*, 2011; Andersen *et al.*, 2017). However, many studies of N uptake rates ignore the organic N component of the soluble soil N pool, though it could be an important N source, especially in northern forests (Lipson & Näsholm, 2001; Kielland *et al.*, 2007; Kranabetter *et al.*, 2007). The more limited body of research on organic N uptake rates suggests that the current *ex situ* measurements of inorganic N uptake alone may not adequately describe competition for N in northern forests. There is also substantial variation in measurements of N uptake rates by N form (Näsholm *et al.*, 2009; Britto & Kronzucker, 2013), which indicates we still lack an understanding of the mechanisms underlying the acquisition of this limiting resource.

Many factors can impact a species' N uptake rate. Environmental factors that influence N uptake rates include N availability (McKane *et al.*, 2002; Li *et al.*, 2015; Kiba & Krapp, 2016), soil temperature (Leffler *et al.*, 2013; Boczulak *et al.*, 2014), soil moisture (Larson & Funk, 2016), season (Socci & Templer, 2011), and soil depth (McKane *et al.*, 2002). As mentioned previously, N uptake rates also vary by the form of N absorbed by plants (nitrate, ammonium or organic forms such as peptides) (Britto & Kronzucker, 2013), through differences in the energetic requirements for assimilating each form (Miller & Cramer, 2004) and by the availability of those forms at a site (Rothstein *et al.*, 2000; Öhlund & Näsholm, 2004).

However, even within similar environmental conditions, uptake rates can still vary among species. These differences can be influenced by biomass carbon (C) allocation, e.g. species with a greater root biomass absorb more N (Leffler *et al.*, 2013), mycorrhizal associations (Turnbull *et al.*, 1996), N uptake physiology or N demand. For example, some species may have reduced physiological capacities to absorb certain forms of N, as with *Picea glauca*, which has atrophied metabolic systems related to nitrate uptake (Kronzucker *et al.*, 1997). Other species differ in the kinetic parameters of N uptake (e.g. V_{max} - the maximum uptake rate for an N form; Hangs *et al.*, 2003; Grassein *et al.*, 2015; Legay *et al.*, 2020). Species also differ in their demand for N, for example, species that have faster growth rates may need to acquire N more quickly to facilitate their high rates of primary productivity (Wright *et al.*, 2004).

In order to improve our understanding of ecologically-significant differences in N uptake among species, and how uptake differs both by species and N forms, it is

important to account for the important sources of variation in N uptake rates. One source of variation that is commonly omitted from studies of N uptake is that of root morphology. Morphological characteristics influence plant access to resources, such as the soil volume that roots are able to explore for a given biomass investment, and thus the amount of nutrients and water available to the plant for exploitation (Bardgett *et al.*, 2014). Root morphological traits, including the ratio of length to mass (specific root length, SRL), root tissue density (RTD), and root diameter, have been shown to correlate to N acquisition (Leffler *et al.*, 2013; Grassein *et al.*, 2015; Moreau *et al.*, 2015; Larson & Funk, 2016; Ma *et al.*, 2018; Legay *et al.*, 2020). Morphology may also be indicative of physiological investment in a root, as certain aspects of root morphology (SRL, diameter) are correlated with root order, or the position of the root within the root system, analogous to stream order (Wells & Eissenstat, 2003). Root order is in turn related to the root's capacity for N uptake (Hawkins *et al.*, 2014).

Though both morphology and mass-specific N uptake rates influence plant competition for N, they are frequently considered independently, which limits our understanding of the ways they may interact to influence whole plant N uptake. Studies of uptake rates that ignore potential variation in N uptake rates due to root morphology could incorrectly attribute differences in uptake rates to physiological differences (e.g. transporter kinetics) rather than morphological differences. For example, if uptake increases with SRL, and two species differ substantially in that trait, their mass-specific uptake rates may differ primarily by differences in surface area rather than in kinetics; yet without measuring morphology the cause may be indistinguishable (Fig. 1.1).

This study sought to improve understanding of N acquisition by determining the influence of morphology on N uptake rates of young individuals of tree species from the boreal-temperate forest ecotone. We focused on characterizing variation in N uptake among species (potentially connected to physiological variation) at the boreal-temperate ecotone because resource competition could play an important role in structuring forests in this ecotone as climate changes and future conditions favor different species. To our knowledge, ours is the first study to compare root morphology to uptake of organic, in addition to inorganic, N in trees. Specifically, we sought to determine (i) if N uptake rates vary with different morphological traits, and (ii) whether N uptake rates vary by species and N form. We hypothesized that (i) N uptake rates would show positive (SRL) or negative (RTD, diameter, RDMC) relationships with root traits because roots with longer, thinner morphologies have higher surface areas for a given mass, allowing them to contact more nutrients for uptake (Fig 1.1 A-D). These roots may also have higher mass-specific uptake rates if plants invest more in N transporters in roots that can explore more soil volume. Additionally, we expected SRL to be the most correlated with N uptake of the traits measured because of its relation to root N content and metabolism (Roumet *et al.*, 2016), which can be indicative of N uptake capacity.

We also expected (ii) that all species would take up more ammonium than nitrate or glycine, consistent with other studies in boreal and other northern systems (Öhlund & Näsholm, 2001; McKane *et al.*, 2002; Hangs *et al.*, 2003), and that the relationship between root traits and N uptake rate would vary among N forms due to differences in the kinetics of root N transporters and energetic differences in uptake and assimilation among N forms (Miller & Cramer, 2004) (Fig 1.1 E-H). Finally, (iii) we expected that species

would differ in N uptake rates due differences in N demand, and that species with greater N demand would show greater uptake rates across N forms and stronger relationships with SRL (higher slopes) than those with lower N demand (Fig. 1.1 E, I-L). Table S1.1 ranks the study species based on traits associated with the leaf economic spectrum (LES) to determine which species should have more N demand, as species with more acquisitive traits should require more N to support their rapid growth (Wright *et al.*, 2004).

To examine the impacts of root morphological traits on N uptake rates we collected individual fine roots (<2 mm) from tree monoculture plots of a common garden study. This common garden is part of the International Diversity Experiment Network with Trees (IDENT) in northern Minnesota, USA (Tobner *et al.*, 2014). We studied seven North American and European tree species: *Quercus rubra*, *Betula papyrifera*, *Pinus strobus*, *Picea glauca*, *Larix laricina*, *Acer platanoides*, and *Acer saccharum*. These species vary in growth rate and mycorrhizal status and include angiosperms and gymnosperms, suggesting they may vary in their morphology and physiology, including their rates of N uptake (see Table 1.1). We quantified the uptake of nitrate, ammonium, and glycine by roots of each species and related uptake rates to measures of root morphology, specifically SRL, average diameter, RTD, and RDMC.

Materials and methods

Sampling location

Root and soil samples were collected from monoculture plots of the IDENT experiment at Cloquet Forestry Center in Cloquet, MN (46° 40’ 46” N, 92° 31’ 12” W,

elev. 382 m, 4.8 °C mean annual temperature, 783 mm mean annual precipitation) (Tobner *et al.*, 2014). IDENT is a forest common-garden experiment that was established in 2010 on a previously forested site with sandy-loam soils (Tobner *et al.*, 2014). The experiment manipulates tree species richness and functional diversity using six pairs of species from genera found in both Europe and North America. Each experimental plot contains 49 trees in a 7 x 7 grid with 40 cm spacing between individuals (2.4 m x 2.4 m plots). The experiment has four replicate blocks, and each species is planted in one monoculture plot per block.

Root and soil sampling and root selection

We obtained excised roots for measurements of N uptake and morphology by collecting root cores from monoculture plots at IDENT. Thus, we controlled for diverse factors that might influence N uptake and morphology such as age of the individual (all trees were within 1-2 years of age of each other) and variation in N availability, light availability, and climate, while including variation in functional diversity. We only sampled in monoculture plots to facilitate identification of roots to species and avoid effects on uptake of growing with heterospecifics. We sampled seven of the total 12 species of trees included in IDENT: *Acer platanoides* and *A. saccharum* on 14 Sept 2018; *Betula papyrifera*, *Larix laricina*, and *Quercus rubra* on 8 Sept 2018; and *Pinus strobus* and *Picea glauca* on 7 Oct 2017 (see Table 1.1 for functional characteristics of these species). We chose these species to capture all of the North American tree species (6 total) and added in the European maple to have another species that associates with

arbuscular mycorrhiza. We sampled three replicate monoculture plots for each species using 5 cm diameter root cores to a depth of 10 cm at two random points that had no herbaceous vegetation or permanent sampling equipment and were at least 80 cm from the plot edge to minimize edge effects and avoid sampling roots from non-target species. Additionally, we cored equidistant between trees in an effort to obtain roots from at least 4 different individuals per core (eight per plot) to minimize the influence of an individual tree on our measurements. The two root cores were composited and sealed in a plastic bag and kept cool on ice for transport to the University of Minnesota laboratory in Saint Paul, MN.

In the lab, we separated the roots from the soil with an 8 mm sieve. The soil was stored in a refrigerator for further analyses. We cleaned the roots through a series of 2-3 nanopure water washes, while keeping the roots cool and moist. We then chose 18 segments of absorptive fine roots from each plot (n=54 per species, 378 total) that were approximately 2 mm or less in diameter, 2-3 cm long, branched, and white or brown in color (these segments of fine roots are hereafter simply referred to as roots). Diameter, branching, and color are all roughly indicative of whether a root is absorptive (Aerts & Chapin, 1999). We completed the uptake incubation within 24 hours of root extraction to ensure roots were still active.

Quantifying nitrogen uptake by depletion

We measured N uptake by immersing the roots in solutions of dissolved N for two hours and measuring the concentration change, after the depletion method of Zerihun &

BassiriRad (2001). Previous studies with similar temperate tree species have shown maximum uptake rates (V_{max}) in concentrations near 100 μM (Zerihun & BassiriRad, 2001), so uptake solutions were made with a concentration of 100 μM N to prevent changes in concentration during incubation from significantly impacting estimates of N uptake rates, and to avoid exposing roots to excessively high concentrations of N. For each of the three N form treatments (glycine, ammonium sulfate, and calcium nitrate), six roots were randomly selected for immersion from each plot sampled so that 18 roots per species were incubated with each N form. We placed each cleaned root in a 1.5 mL microcentrifuge tube containing 1 mL of a 100 μM-N solution of either glycine, ammonium sulfate, or calcium nitrate. The roots were incubated for two hours at room temperature (ca. 22°C), which is similar to the duration used in other studies and balances the need to allow adequate time for nutrient depletion to be measurable without exhausting the energy reserves of the excised roots (Lucash *et al.*, 2007). Controls for contamination included tubes incubated with solution but without roots. After the incubation, we removed the roots from the uptake solutions and stored them in a refrigerator. The solutions were frozen at -20 °C until analysis for N concentration.

The change in N concentration that occurred during incubation was measured via colorimetric analyses. For all analyses, absorbances were measured on a BioTek Synergy H1 microplate reader (Winooski, Vermont, USA) and then converted to concentrations using standard curves. We analyzed glycine concentrations using a modified ninhydrin protocol (Jones *et al.*, 2002). Prior to analysis, we added 25 mg magnesium oxide (MgO) to each microcentrifuge tube and let them sit open overnight to remove any ammonium (as the pH increases, ammonium gives up a proton and volatilizes as ammonia) that the

roots may have exuded that would impact our measurement of the amino acid concentration (the ninhydrin reagents react to NH_4^+ with reduced sensitivity). We measured the change in volume to account for any loss of solution to evaporation and adjusted concentrations accordingly in our analysis. After volatilizing the ammonia, we combined 100 µL of glycine solutions with 75 µL of ninhydrin reagent (0.3g hydrindantin dihydrate and 2g ninhydrin dissolved in 75 mL DMSO, with 25 mL 4M sodium acetate buffer (pH 5.2) added immediately prior to analysis) in 96 deep well plates sealed with microplate covers and incubated them in an 80 °C water bath for 30 minutes (Jones *et al.*, 2002; Friedman, 2004). Once the samples had cooled, we added 825 µL of 50% ethanol as a stabilizing agent and transferred samples to 96 well microplates and read the absorbance at 570 nm.

Ammonium concentrations were analyzed via the indosalicylate method (Verdouw *et al.*, 1978). Briefly, 80 µL of the uptake solution were combined with 60 µL of sodium salicylate reagent (sodium salicylate, sodium citrate, sodium tartrate dibasic dihydrate, and sodium nitroprusside) and 60 µL of bleach solution (150 mg/L sodium hypochlorite in 1 M NaOH) in clear microwell plates and incubated for 2-4 hours in the dark; following incubations, the absorbances were read at 650 nm. Finally, nitrate concentrations were measured using the vanadium chloride method (Doane & Horwáth, 2003). We combined 100 µL of uptake solutions with 100 µL of vanadium (III) chloride reagent (100 mL of 1M HCl, 200 mg of sulfanilamide, 100 mg of N-(1-naphthyl)-ethylenediamine dihydrochloride, 50 mL 1 M HCl, 400 mg of vanadium (III) chloride) in microwell plates and incubated them in the dark for 5 hours before reading absorbances at 540 nm.

We calculated nitrogen uptake rate on a dry mass basis for each root used. We first converted pre- and post-uptake N concentrations to mg N using the respective solution volumes. The post-uptake mg N was subtracted from pre-uptake mg N to give total mg N absorbed. To account for loss of N through adsorption of uptake solution to roots upon removal from the solution, the volume change was multiplied by the final N concentration to give an estimate of mg N removed. This was then subtracted from total mg N absorbed. We standardized the resulting value by root dry mass and divided by the incubation time to give a rate of N uptake (mg N g^{-1} DW hr^{-1}).

Quantification of morphology

We measured four different morphological characteristics of the roots. First, we weighed roots within 10 days of extraction to get their fresh mass on a Sartorius Pro 11 microbalance. We stored them at -20 °C until they were scanned using a MicroTek ScanMaker 9800XL scanner and WinRHIZO version 2005a,b software (Regent Instruments Inc, Quebec). We used the WinRHIZO image analysis software to analyze the scans for root diameter, length, and volume. We then oven dried the roots at 60 °C for 48 hours and weighed them on the microbalance to obtain dry mass. We calculated SRL by taking the ratio of the length and dry mass. Average diameter was taken directly from the scan output. While we are confident that none of our roots had a diameter greater than 2 mm, some of the diameters reported by WinRHIZO were greater than 2 mm. WinRHIZO is sometimes unable to distinguish between different branches of very fine roots (i.e. treating them as a single branch), which could explain this result. We

calculated RTD from the ratio of the dry mass and volume. RDMC was calculated from the ratio of the dry mass to the fresh mass.

Statistical Analyses

We examined correlations between root morphological traits to determine whether we should include multiple traits in the same linear model. We log-transformed all trait variables because all traits were found to best fit a lognormal distribution (*fitdist* function from *fitdistrplus* R package v. 1.0-14). Then we calculated Pearson correlation coefficients between each pair of traits (R package *Hmisc* v. 4.3-0, *rcorr* function). We also compared differences in traits across species to see which traits varied most among species (a possible indication that those traits are more likely to contribute to differences in uptake rate). For each trait, we performed a generalized least squares analysis (*gls* function in *nlme* R package v. 3.1-147), weighted by species, with the variance given the form of $\varepsilon_{ij} \sim N(0, \sigma_j^2)$ $j = 1, \ldots, 7$, where j refers to an individual species. We assessed the overall significance of species using the *Anova* function with type III sums of squares (*car* package v. 3.0-6). Post-hoc analyses were conducted via the estimated marginal means method (*emmeans* package v. 1.4.4), using the Tukey method to adjust for multiple tests.

To test for differences in uptake by species and N form, we began by using GLS to weight the variance by species or N form if needed. Before analysis, uptake rates were log-transformed after adding the minimum uptake value + 0.1 to remove negative values. However, examining the diagnostic plots showed that weighting the variance of uptake

values by species and N form did not improve normality or homogenize variance. Therefore, we summarized these patterns using descriptive statistics.

We next compared models without traits to the models that included root traits by fitting GLS models with species, N form, and trait and weighting them by species and root trait. We found the best model for each root trait using backwards model selection with the *stepAIC* function (*MASS* package v. 7.3-51.5). Models were fit for each trait individually, as pairwise correlations showed that all traits were correlated with at least two other traits. SRL was the most strongly correlated to other root traits (Table S1.2), including RTD and diameter (r = 0.86, p < 0.0001, r = -0.83, p < 0.0001, r = -0.66, p < 0.0001) but not RDMC (r = -0.04, p = 0.54). All other traits showed weak (r < 0.5) correlations with each other. We then compared models of each root trait using the ΔAIC and ΔBIC values to evaluate which traits had the strongest explanatory power for N uptake rates. We then proceeded to test our various hypotheses (Fig. 1.1) using post-hoc comparisons with the *emmeans* package, using the *emmeans* and *emtrends* functions. We compared species differences at the mean trait value and looked for differences in the relationship of each species and N form with the trait by testing for differences in the slope. We focus our discussion on the trait with the most explanatory power (SRL) given that it was strongly correlated with the other traits.

Model assumptions of normality, linearity, and homogeneity of variance were checked with graphic analyses. These graphs indicated that the model assumptions were met for all models except for those examining N uptake by species and N form without traits. We used graphical analyses to test assumptions because statistical tests for

normality can be very sensitive at larger sample sizes and tests for homogeneity of variance are also sensitive to non-normal data (Zuur *et al.*, 2009). For all GLS models, we first identified the ideal variance structure by fitting a full model (all fixed effects and interactions) with different variance structures using the "REML" fit method and comparing models with the *anova* function in R (Zuur *et al.*, 2009). If two potential structures were similar, we examined plots of residuals and used the structure that adhered more closely to model assumptions. After finding the ideal variance structure, we fit the full model using the maximum likelihood method and determined whether any interaction terms should be dropped from the model using model selection (*stepAIC* in *MASS* package). After determining which fixed effects to drop (if any) we refit the model with the "REML" method and tested the significance of the fixed effects using the *Anova* function with type III sums of squares (*car* package). We fit all models with effects coding for categorical variables rather than the default dummy coding since there was no control species or N form. All analyses were conducted in R version 3.6.2.

Results

Root morphological traits are correlated and vary by species

Species differed from one another in their morphological traits, though SRL and RTD varied more among species than other traits. For example, while species accounted for a substantial amount of variation for all morphological traits (Table 1.1, Table S1.3), more pairwise contrasts between species were significant for SRL and RTD (14 each) and diameter (12) compared to RDMC (1). The maples tended to have similar trait values

compared to the other five species; they had SRL values from just over 2 to almost 6 times higher than the SRL for the other five species (Table 1.1). Similarly, both maples had substantially lower RTD than the other species, with values about ⅓- ⅔ of the RTD of the remaining species. Whereas *B. papyrifera*, *L. laricina*, *P. glauca*, and *Q. rubra* grouped together in terms of similar values for RTD and SRL, *P. strobus* had the lowest SRL and highest RTD of all other species.

Some species exhibited greater intra-specific variation than others in their morphological traits. Both *Q. rubra* and *P. strobus* tended to have more variable traits than the other species, as shown by the estimates of σ for the GLS weights (Table S1.4). In contrast, the maples exhibited the lowest variation in their root traits, with *B. papyrifera*, *L. laricina*, and *P. glauca* generally intermediate.

Relationship of root morphological traits with N uptake rates

To test our hypotheses about trait relationships with N uptake rates, we evaluated the best fit GLS models and examined the direction of the relationships. All the root morphological traits, considered one at a time, were significant predictors of N uptake rates, and the direction of those relationships matched our hypotheses. In model selection, each root morphological trait was retained in the best fit model, even after accounting for variation in uptake rates by species and N form ($p < 0.01$ for all traits, Tables 1.2, 1.3, S1.5). Additionally, all models included an interaction term between species and N form. Models also included additional interactions between the root trait and species and/or N form except for the RDMC trait (Table 1.2, S1.5). The slopes of the relationships

between N uptake rates and SRL were positive when averaged across species, though this relationship varied by species and N form (Fig. S1.1, Table 1.4). However, the direction of this relationship was consistent for all species and N forms except *A. saccharum* (Table 1.4). The remaining traits (RTD, diameter, RDMC) all showed negative relationships between N uptake and increasing values of the morphological trait (Table S1.6), which remained consistent when looking at the relationships for individual species for RTD and diameter except for *A. saccharum* for RTD.

We compared the best fit models for all traits to determine which trait was most predictive of N uptake rates, as well as which model to use to compare differences in uptake rates by species and N form. We determined which trait(s) best explained variation in N uptake rates using models that included all 2-way interactions between trait, N form, and species predictors, as well as the best model from our model selection procedure. In both cases, models that included SRL had much lower AIC and BIC values than models fit with all other traits. For example, the difference in AIC values between the best model with SRL and the best model with RTD was -132.7 (Table 1.2). This suggests that SRL is the strongest predictor of N uptake of the traits measured. Given the correlation between traits, and the much lower AIC and BIC values for models of N uptake with SRL, we focus primarily on interpreting N uptake in the model with SRL.

Differences in N uptake rates by species and N form

The best model for N uptake with SRL, N form, and species as predictors included all 2-way interactions. As mentioned previously, the relationship of SRL with N

uptake was strong and positive, but there were also significant interactions of SRL with both species and N form (Fig. 1.2). While the magnitude of the SRL-N uptake rate relationship varied by species (Table 1.3), this significant relationship was primarily driven by two species (Fig. 1.2). For example, *L. laricina* and *A. saccharum* showed slopes near 0 ($\beta = 0.040$, $t = 1.80$, $p = 0.077$; $\beta = -0.034$, $t = -0.63$, $p = 0.53$, respectively), while the remaining species all had significant positive slopes (Table 1.4). Accordingly, the only significant difference in slopes between species come from the contrasts between species on the extremes: *A. saccharum* had a significantly lower slope than *A. platanoides* and *P. glauca*, the species with the greatest slopes, while *L. laricina* had a significantly lower slope than *P. glauca* (Table 1.4). The remaining species all had relatively similar slope values. When looking at the relationship of SRL and N uptake by N form, the slope for NH_4^+ (0.133 ± 0.02 SE) was greater than for both glycine (0.102 ± 0.02 SE) or NO_3^- (0.065 ± 0.023 SE). However, the uncertainty around these estimates only distinguishes the difference between NH_4^+ and NO_3^- (diff = 0.069, $t = 3.25$, $p = 0.008$, Table 1.4).

In addition to comparing the relationship of N uptake rate and SRL across species and N forms, we also examined the mean uptake rates by species and N form. Mean uptake rates for most species were the highest for ammonium, while glycine uptake rates were the most variable among species (-0.005 - 0.076 mg N g^{-1} DW hr^{-1}) and nitrate uptake rates were lowest (0.016 - 0.025 mg N g^{-1} DW hr^{-1}, Table 1.5, Fig. 1.3). Mean ammonium uptake across species (0.056 mg N g^{-1} DW hr^{-1}) was almost three times greater than mean nitrate uptake (0.020 mg N g^{-1} DW hr^{-1}) and mean glycine uptake was nearly two times greater (0.035 mg N g^{-1} DW hr^{-1}). *A. platanoides* had the highest mean

uptake (0.054 mg N g^{-1} DW hr^{-1}), while *A. saccharum* had the lowest mean uptake overall (0.020 mg N g^{-1} DW hr^{-1}). *A. platanoides* also had the highest uptake rate for ammonium (0.074 mg N g^{-1} DW hr^{-1}) and glycine (0.073 mg N g^{-1} DW hr^{-1}), while *A. saccharum* had the second lowest uptake for ammonium (followed by *L. laricina*) and was the only species with no net uptake of glycine.

Discussion

Trait-uptake relationships

We investigated whether root morphological traits influenced N uptake rates and if the relationship depended on species and N form to improve our understanding of plant N acquisition. Nitrogen uptake rates were consistently correlated with root morphological traits in the directions hypothesized (Fig. 1.1A-D), and SRL explained more variation in uptake rates than the other three traits by a large margin. This finding is consistent with our prediction that roots with longer, thinner morphologies are more active in acquiring N. Though few studies have examined both morphology and N uptake rates in trees, many studies have looked at relationships for N uptake rates and SRL in other growth forms and have found similar positive relationships to those reported here (Larson & Funk, 2016; Hong *et al.*, 2018; Legay *et al.*, 2020). Additionally, the study by Hong et al. (2018) showed a negative relationship between diameter and N uptake rates for NH_4^+ and NO_3^-. While some studies have failed to find relationships between morphological traits and N uptake, those studies considered relationships on different spatial scales (i.e. whole root system as in Ravenek et al. (2016) vs. root segments in this study) or measured

uptake rates and morphological traits on separate roots (Ma *et al.*, 2018) possibly obscuring relationships.

The stronger relationship of N uptake with SRL compared to the other root traits could be due to several factors. First, of the traits measured, SRL is the most predictive of the amount of soil volume a root is able to explore for a given mass investment and therefore the amount of nutrients and water available to the root for uptake. Though our study was done in solution, not soil, the surface area of the roots used would still limit their overall rate of uptake. Since a solution allows for more rapid rates of diffusion, greater area for absorption (i.e. higher SRL) should be even more important in soil where diffusion is more limited and the soil volume explored per unit mass plays a stronger role in determining total N uptake (Hodge *et al.*, 1999; Bonifas & Lindquist, 2010). Second, higher SRL has been shown to correlate to higher hydraulic conductivity, or a greater rate of movement of fluid through a root (Eissenstat & Achor, 1999). Faster flow through a root is critical to uptake capacity in nutrient-rich soils, where uptake is in part determined by mass flow or the movement of the soil solution towards the root induced by transpiration, and therefore in part controlled by the capacity of the roots to conduct solution (Oyewole *et al.*, 2015). While in aboveground tissues, tissue density has been commonly used as a predictor of hydraulic conductivity, evidence suggests that the relationship between density and conductivity is not as strong in belowground organs, supporting our finding that RTD was not as strongly correlated with uptake as SRL (Pratt *et al.*, 2007; Lachenbruch & Mcculloh, 2014). Third, SRL is more consistently correlated to root order than the other traits and evidence has shown that root order is predictive of uptake. Lower root orders, the farthest from the shoot, are the most absorptive and higher

orders, the closest to the shoot, are the least absorptive (Wells & Eissenstat, 2003; Rewald *et al.*, 2011; Hawkins *et al.*, 2014). The more absorptive lower order and younger roots consistently have higher SRL values (Rewald *et al.*, 2011; McCormack *et al.*, 2012). Thus, our finding that SRL controls uptake most strongly is consistent with current knowledge of both the mechanical relationship between root morphology and uptake and what is signified by morphology, in terms of the role a root plays in a root system.

Differences between N forms in uptake and trait-uptake relationships

As predicted, all species took up more ammonium than nitrate or glycine and this trend was consistent across all values of SRL. Rates of uptake for nitrate were the lowest of all N forms for all species in our study, in keeping with our prediction (Fig. 1E-H) and findings from other studies in boreal and other northern systems. Temperate and boreal forest species from multiple continents tend to take up N more quickly as organic N or NH_4^+ rather than NO_3^- (Rothstein *et al.*, 2000; Öhlund & Näsholm, 2001; McKane *et al.*, 2002; Hangs *et al.*, 2003; Persson *et al.*, 2006; Scott & Rothstein, 2011; Gruffman *et al.*, 2014; Liu *et al.*, 2017), although one study found higher uptake rates for NO_3^- than NH_4^+ or ON in *P. sitchensis* (Metcalfe *et al.*, 2011). Our uptake rates are also comparable to those reported for similar N forms and species in studies that used similar methods (Scott & Rothstein, 2011; Liu *et al.*, 2017).

Furthermore, N uptake rates increased more with SRL for ammonium, while nitrate uptake was the least impacted by variation in SRL. The larger impact of SRL for

different N forms is illustrated by Fig. 1.1H, and may result from a few potentially interacting mechanisms. Differences in the SRL- N uptake rate relationship by N form could be influenced by the kinetics of the transporters for different N forms. While some plants may simply have lower capacity for NO_3^- (Min *et al.*, 1998), low external NO_3^- concentrations can result in lower observed V_{max} values, because the main NO_3^- transporters used at low NO_3^- levels tend to have lower V_{max} than the transporters used at higher concentrations (Crawford & Glass, 1998; Miller & Cramer, 2004). Additionally, roots may simply have fewer transporters for NO_3^- when there is low NO_3^- availability because the induction of root NO_3^- transporters with higher V_{max} will only be triggered by higher NO_3^- concentrations (Crawford & Glass, 1998 and refs 23, 24 therein). Fewer transporters for NO_3^- would constrain the ability of roots to take up NO_3^- when available, and would be exacerbated at higher SRL.

Low NO_3^- availability is likely a factor in our study, as previous measurement of net nitrification rates in the same plots used in our study found low and mostly negative rates (L. Williams, unpublished data), and soil NO_3^- concentrations were low at the time of our sampling (unpublished data). Thus, a lack of induction of NO_3^- transporters could be constraining NO_3^- uptake rates for our study species even at high SRL. Further tests would need to involve induction of NO_3^- transporters to determine whether the potential V_{max} for NO_3^- and the other N forms actually differ.

Different forms of N also have different costs for their uptake and assimilation into plant biomass. The variable uptake-SRL relationships for each N form may reflect mechanisms influencing uptake rates. Nitrate assimilation requires energy to reduce NO_3^-

to NH_4^+ so it can be attached to a carbon skeleton. Thus, NO_3^-, uptake is more costly than simple NH_4^+ assimilation (Miller & Cramer, 2004). However, NH_4^+ uptake requires quick attachment to a C-skeleton because it is toxic to the cell in large quantities (Britto & Kronzucker, 2002), and thus there may be times when C is limiting to NH_4^+ uptake. Amino acid (AA) uptake is a bit more complicated as the cost can vary by the type of AA. For example, some AAs can be transferred to the shoot for reduction (i.e. glycine), indicating they may not have the same toxicity impact of NH_4^+, while others have been shown to inhibit plant growth at higher concentrations like NH_4^+ (Näsholm *et al.*, 2009). However, once taken up, AAs are generally metabolized into other AAs, which may be less costly than incorporating NH_4^+ or NO_3^- into those AAs (Franklin *et al.*, 2017) and could favor their uptake in certain situations.

Given that the roots in our study had limited energy supply due to their excision, the more energetically costly N forms (for uptake or assimilation) could have depleted the roots' energy supply more quickly and resulted in lower overall uptake. These differences in costs could impact the relationship between uptake and SRL among forms of N. For example, NO_3^- uptake may deplete a root's energetic resources more quickly than NH_4^+ uptake, and the root would be able to take up less total N when supplied with NO_3^- versus NH_4^+. As a root's uptake potential increases (i.e. at higher SRL), differences in observed uptake rates would become larger as the less costly form could still be taken up by the root at the point when the more costly form had expended all of the root's energy resources.

Species differences in uptake and trait uptake relationships

While we found that differences in uptake rates by N form were relatively consistent across species, interspecific differences in uptake rates depended on N form, were less predictable, and the species with the highest uptake rates did not entirely match our hypothesis. We expected that species might vary in uptake rate by form, but that species with greater N demand would be relatively consistent in having high uptake rates across forms. However, the two species with the most acquisitive leaf traits (used as an indicator for plant N demand, Table S1.1, (Wright *et al.*, 2004; Reich, 2014)), differed in their uptake rates. *A. platanoides* had the highest uptake rates for NH_4^+ and glycine but had the lowest mean uptake rate for NO_3^-, and *B. papyrifera* had the second highest uptake rate for NH_4^+ but the fifth highest uptake rate for glycine. Additionally, *A. saccharum* had more acquisitive leaf traits than many species in this study but exhibited the lowest uptake rates of all species.

Complexity in the physiological and environmental aspects of N uptake, and the species-specific variation in traits influencing N uptake produce a relatively complex conceptual model for variation in N uptake. The variation in uptake rates by N form could reflect differences in plant strategies for acquiring N that may not be reflected in their leaf traits. For example, *A. saccharum* may lack the capacity for taking up glycine, as not all species have documented uptake of organic N forms (Näsholm *et al.*, 2009), and certain species may be better adapted to take up one form over another due to variation in their energetic costs for uptake and assimilation (i.e. NH_4^+ over glycine). However, it is interesting that the other maple in this study had the highest uptake rate of

all species for glycine. It is also possible that there was variation in each species' relative N demand might be another factor influencing uptake, since differing N demand can cause species to up- or downregulate N uptake rates (Britto & Kronzucker, 2013), in a way that does not match their relative acquisitiveness. Luxury consumption of N is another complicating factor. Some species may be taking up N that they do not immediately need as a form of luxury consumption (Chapin, 1980), which may obscure differences in uptake rate that would otherwise be caused by variation in overall plant N demand. Species may also have different thresholds for external N concentrations that trigger the transcription of root transporters that have higher V_{max}. Even though our experiment was conducted in a common garden, enough time has elapsed since establishment for species to influence plot-level N availability. Thus, we could be comparing uptake rates of different transporters (High affinity vs. Low affinity), which have different kinetics (Miller & Cramer, 2004). Experiments that are able to manipulate N status and measure uptake rates, transporter abundance, and transporter type would help further elucidate the mechanisms underlying species differences in uptake of different N forms.

In contrast to mean uptake rates, the relative ranking of species in the slope of the SRL - N uptake relationship did not vary by N form, though the species with the greatest slopes did not necessarily match those identified in our original hypothesis. This relationship is best visualized by the hypothesis presented in Fig. 1.1L, as species varied in both the slope and intercept of the SRL-uptake relationship, and all species except *A. saccharum* showed a positive slope. It should be noted that most species had relatively similar slopes and the differences in slopes were primarily driven by species differing

from the two species with the lowest slopes, *L. laricina* and *A. saccharum* This result is consistent with other studies that have found similar trait-uptake relationships across species (Hong *et al.*, 2018). However, the species with the greatest slopes were not the ones with the most acquisitive traits, in contrast to our hypothesis: while *A. platanoides* had the highest slope and relatively acquisitive traits, the next two highest slopes belonged to species that had the most conservative leaf traits. One potential explanation for the unexpected findings for some species could be due to the influence of phenology on the N uptake rates and thus the SRL-N uptake. Field studies of N uptake in trees (Li *et al.*, 2016) and herbaceous species (McKane *et al.*, 2002) have shown that uptake rates can vary by season, with some species taking up more N at different times of year than their competitors, while others have relatively constant uptake rates across seasons. This could be one reason for the lack of a relationship between SRL and N uptake rate in *A. saccharum*. A study by Socci and Templer (2011) found that at the N concentration used here (100 µM), *A. saccharum* took up less ammonium in the fall than in mid-summer, and had no significant uptake of nitrate. Given these low uptake rates, N uptake would not be expected to increase much with SRL. Similarly, the species with the strongest relationships may be particularly active at this time of year.

Another explanation for the unexpected pattern in slopes could be that species vary in how quickly they respond to changes in N availability, which can change rapidly (Jackson *et al.*, 1972; Kielland *et al.*, 2007). While morphology may not be particularly plastic in response to increases in N availability (Eissenstat *et al.*, 2015; Chen *et al.*, 2016; Weemstra *et al.*, 2017), but see also (Ostonen *et al.*, 2007), some species may be able to more quickly up- or down-regulate transporters based on those short-term

fluctuations in N supply, while others may take longer to respond (Kronzucker *et al.*, 1997). If we sampled roots at a time when N availability was changing, we could be capturing up-regulation for some species while others may be lagging, and lower overall uptake rates limits the potential slope of the uptake-SRL relationship. While we tried to control for that by using our common garden study, experimental studies that can limit spatial and temporal heterogeneity in N availability could augment our understanding of variation in the trait-uptake relationship by species.

An important implication of the differences in slopes between SRL and uptake rates among species (and N forms) is that morphological differences were not the only reason species differed in uptake rates. Species with the highest slopes were not necessarily the ones with the highest uptake rates (e.g. *P. glauca*). This result can be explained by a combination of some species having higher SRL on average even with lower slopes (e.g. *B. papyrifera*), as well as higher baseline uptake rates (higher intercepts). Thus, for a given species, a combination of strategies could increase uptake rates in competition for N. Species can invest more in transporters per length root (increase intercept and/or slope), alter root morphology to allocate mass to increase surface area available for uptake (increase overall uptake), or a combination of the two. Even though more research on differences in the regulation and kinetics of root N transporters will help augment our findings, it remains clear that examining morphology can help us predict N uptake rates across species.

Considerations for extrapolating results

As described above, root morphology and physiology, along with chemical differences in N forms, can lead to the patterns we observed in our study. However, extrapolating our findings across species and environmental conditions should consider that the strength, slope, and intercept of this relationship will likely vary by species or N form and the external conditions. For example, if we had sampled roots from a cooler location there may have been lower ammonium availability relative to organic N. Differences in the relative availability of organic N could have resulted in species being more primed for uptake of amino acids rather than ammonium and as a consequence we could have observed more species with higher uptake rates for amino acids. Additionally, our uptake rates may be lower than those reported for measurements on intact roots because the carbon supply to roots has been cut off and N uptake requires active transport (Lucash *et al.*, 2007). However, greater uptake capacity in situ could actually enhance the separation of species and the SRL-N uptake relationship we observed by providing the energy to fully realize species differences in N uptake capacity. The excision of fine roots also necessitates severing the extraradical mycelia of mycorrhizal fungi, so we did not capture their contribution to N uptake in this study. The importance of mycorrhizal fungi for N uptake in many species (Nave *et al.*, 2013) means our measured uptake rates may be less representative for species that rely heavily on mycorrhizal fungi for N uptake. However, trees still acquire N outside of that symbiosis, so the factors that influence non-mycorrhizal root uptake remain an important component of whole plant N acquisition. An additional caution for extrapolation is that the roots of the tree species studied here represent only the high tail of the distribution of SRL values in plants (Ma *et al.*, 2018).

Studies with a broader sample of SRL values are needed to discern whether these patterns are more or less pronounced for species with higher SRL than those used in our study.

Conclusions and Future Directions

Our consideration of morphological traits in the analysis of N uptake rates in seven tree species and for three N forms showed consistent relationships of morphological traits, especially SRL, with N uptake. We demonstrate that even on small sections of root there was enough variation in the morphological traits we measured to influence N uptake rates. In trying to address questions solely about root physiology it is therefore important to account for variation in root morphology. Ignoring morphology could result in incorrectly attributing differences in uptake rates to physiological differences (i.e. V_{max}, transporter density), when in part they may be due to differences in morphology. This error could be especially important in studies looking at differences in uptake rates among N forms or among species across gradients, where root morphology may vary. However, as we could not determine whether the relationship of N uptake and SRL was due to increased surface area, differences in uptake kinetics, or transporter density, more studies that examine the mechanisms underlying this correlation are necessary.

Our results also indicate that to accurately measure and model N acquisition likely requires careful consideration of the morphology of the roots being measured. Incorporating root traits into models has been shown to be necessary to accurately recreate the observed spatial patterns in plant N uptake, but have yet to be incorporated

into earth-system models more broadly (Zhu et al. 2016). Furthermore, our results suggest that measuring morphology, particularly SRL, may provide a faster and simpler way to account for variation in N uptake rates in roots of different ages, orders or anatomy than attempting to measure those attributes directly, given the correlation of SRL with many of those root properties (e.g. root order, Pregitzer et al., 1997). It is extremely time intensive and sometimes infeasible to determine root order, assess root age, or measure anatomical traits related to N uptake, so significant time and resources could be saved if more easily measurable traits such as SRL can be used as a proxy. For morphological traits like SRL to be used in that manner will require more studies that measure uptake and morphology to examine how consistent the relationship between SRL and N uptake rates are across species and N forms, and whether the relationship holds at different times of year or in different environmental conditions. We would expect that species with more seasonal N acquisition would exhibit greater variation in the relationship between N uptake and morphology, and that environmental conditions that impact N availability (soil moisture, temperature) would also impact that relationship. Even though we expect that the relationship of N uptake and morphological traits may vary, our study has shown that ignoring morphology can obscure inference on the reasons for species and N form differences in N uptake rates. Therefore, by more explicitly accounting for variation in morphological and physiological trait variation within roots, we can more thoroughly understand N acquisition in plants and how they compete for and successfully acquire limiting nutrients.

Table 1.1. Study species and their functional and root morphological traits. Morphological traits were measured on the same root segments used for measuring N uptake rates and are shown as mean ± SE*. Differences among species are signified by different letters and were determined by GLS models. Species are ordered from lowest to highest SRL, which also orders them into groups by mycorrhizal type (EM first 5 species, AM last 2 species).

Species	Group	Mycorrhizal type	Origin	SRL (m g^{-1})	RTD (g cm^{-3})	Diameter (mm)	RDMC
Pinus strobus	Gym	EM	North American	6.32 ± 0.72 ^{A}	0.106 ± 0.0051 ^{A}	1.73 ± 0.067 ^{A}	0.294 ± 0.011 ^{ABC}
Betula papyrifera	Ang	EM	North American	9.64 ± 0.78 ^{B}	0.0633 ± 0.0041 ^{B}	1.73 ± 0.726 ^{A}	0.303 ± 0.013 ^{ABC}
Larix laricina	Gym	EM	North American	10.0 ± 0.83 ^{B}	0.0612 ± 0.0029 ^{B}	1.65 ± 0.053 ^{A}	0.275 ± 0.0067 ^{ABC}
Picea glauca	Gym	EM	North American	11.2 ± 0.94 ^{B}	0.0577 ± 0.0042 ^{B}	1.65 ± 0.04 ^{A}	0.267 ± 0.0075 ^{B}
Quercus rubra	Ang	EM	North American	15.1 ± 1.71 ^{B}	0.0864 ± 0.01 ^{B}	1.43 ± 0.048 ^{B}	0.306 ± 0.012 ^{ABC}
Acer saccharum	Ang	AM	North American	26.6 ± 1.37 ^{C}	0.0339 ± 0.0022 ^{C}	1.30 ± 0.027 ^{B}	0.295 ± 0.0081 ^{ABC}
Acer platanoides	Ang	AM	European	28.0 ± 1.05 ^{C}	0.0305 ± 0.0013 ^{C}	1.28 ± 0.02 ^{B}	0.303 ± 0.0081 ^{C}

*n = 54 for *P. strobus*, *L. laricina*, and *A. platanoides*; n = 53 for *P. glauca*; n = 52 for *B. papyrifera*, *Q. rubra*, and *A. saccharum*

Abbreviations: SRL = specific root length; RTD = root tissue density; RDMC = root dry matter content; Gym = gymnosperm; Ang = angiosperm; EM = ectomycorrhizal; AM = arbuscular mycorrhizal

Table 1.2. Comparing models of N uptake rates by for each root morphological trait. The model formula of the best GLS model for each trait is given alongside the AIC/BIC values for each model. The ΔAIC/ΔBIC values are shown relative to the model with SRL, which had the lowest AIC/BIC values of all models.

Trait	Model Formula (Best Model)*	df	AIC	BIC	ΔAIC	ΔBIC
SRL	log(SRL) + N form + Species + Block + N form * Species + N form * log(SRL) + Species * log(SRL)	40	-483.3	-326.6		
RTD	log(RTD) + N form + Species + N form * Species + Species * log(RTD)	38	-350.6	-201.8	-132.7	-124.8
Diameter	log(Diameter) + N form + Species + Block + N form * Species	32	-309.5	-184.2	-173.8	-142.4
RDMC	log(RDMC) + N form + Species + Block + N form * Species	32	-311.5	-186.2	-171.7	-140.4

*all models included weights of the form $var(\varepsilon_{ij}) = \sigma^2_j * |log(trait)_{ij}|^{2\delta}$

Table 1.3. Best model of N uptake rates with SRL**.** Results of the best GLS model for N uptake (ln(uptake rate + 0.1)) with SRL, N form, Species, and Block.

Predictor	**df**	χ^2	**p-value**
(Intercept)	1	1253	< 0.0001
Species	6	30.8	< 0.0001
N form	2	0.1	0.9740
Specific Root Length	1	31.1	< 0.0001
Block	2	7.3	0.0266
Species * N form	12	21.9	0.0387
Species * Specific Root Length	6	22.4	0.0010
N form * Specific Root Length	2	11.5	0.0032

Table 1.4. Slopes of SRL – N uptake relationship by species and N form. Slope estimates and the 95% confidence interval come from the best fit model for SRL and N uptake in Tables 1.2 and 1.3 and are reported on the log scale. Differences in slopes are compared across species or across N form as there was not a significant 3-way interaction between species, N form, and SRL: letters next to the species name indicate the results of pairwise contrasts showing which species have similar slopes ($p > 0.05$), while the † and ‡ are used to signify differences between N forms.

	N Form Average		**Glycine†‡**		**Ammonium‡**		**Nitrate†**	
Species	**Slope**	**95% CI**	**slope**	**95% CI**	**slope**	**95% CI**	**slope**	**95% CI**
Average	0.100	0.064 – 0.125	0.102	0.062 - 0.141	0.133	0.093 - 0.173	0.065	0.019 - 0.110
P. strobus [AB]	0.095	0.072 - 0.118	0.097	0.069 - 0.124	0.128	0.103 - 0.153	0.06	0.019 -0.101
B. papyrifera [AB]	0.072	0.034 - 0.110	0.074	0.031 - 0.117	0.105	0.062 - 0.149	0.037	-0.007 - 0.081
L. laricina [A]	0.04	-0.004 - 0.084	0.041	-0.007 - 0.089	0.073	0.026 - 0.120	0.004	-0.048 - 0.057
P. glauca [BC]	0.131	0.094 - 0.169	0.133	0.093 - 0.173	0.165	0.124 - 0.206	0.096	0.048 - 0.145
Q. rubra [AB]	0.07	0.010 - 0.130	0.072	0.009 - 0.134	0.103	0.040 - 0.167	0.035	-0.030 - 0.099
A. saccharum [C]	-0.034	-0.145 - 0.076	-0.033	-0.145 - 0.079	-0.001	-0.112 - 0.110	-0.07	-0.184 - 0.045
A. platanoides [AB]	0.324	0.119 - 0.530	0.326	0.120 - 0.533	0.358	0.151 - 0.565	0.289	0.083 - 0.496

Table 1.5. Mean uptake rates by species and N form. Overall (across N forms) mean uptake rates as well as N form specific uptake rates ± SE are shown for each species. The last row shows the across-species mean uptake rate for each N form.

Species	**Overall Mean Uptake Rate (mg N g^{-1} DW hr^{-1})**	**Ammonium uptake (mg N g^{-1} DW hr^{-1})**	**Glycine uptake (mg N g^{-1} DW hr^{-1})**	**Nitrate uptake (mg N g^{-1} DW hr^{-1})**
P. strobus	0.0363 ± 0.0049	0.0510 ± 0.0108	0.038 ± 0.00846	0.0198 ± 0.0030
B. papyrifera	0.0382 ± 0.0048	0.0662 ± 0.0106	0.0312 ± 0.00501	0.0183 ±0.0027
L. laricina	0.0308 ±0.0029	0.0369 ± 0.0068	0.0327 ± 0.00392	0.0227 ± 0.0035
P. glauca	0.0463 ± 0.0048	0.0587 ± 0.0089	0.0542 ± 0.00724	0.0247 ± 0.0063
Q. rubra	0.0338 ± 0.0064	0.0586 ± 0.0114	0.0226 ± 0.0139	0.0196 ± 0.0041
A. saccharum	0.0200 ± 0.0058	0.0459 ± 0.0077	-0.0054 ± 0.0115	0.0195 ± 0.0067
A. platanoides	0.0542 ± 0.0082	0.0742 ± 0.0127	0.0726 ± 0.0183	0.0159 ± 0.0025
Mean	--------	0.0559 ± 0.0038	0.0353 ± 0.0045	0.0200 ± 0.0016

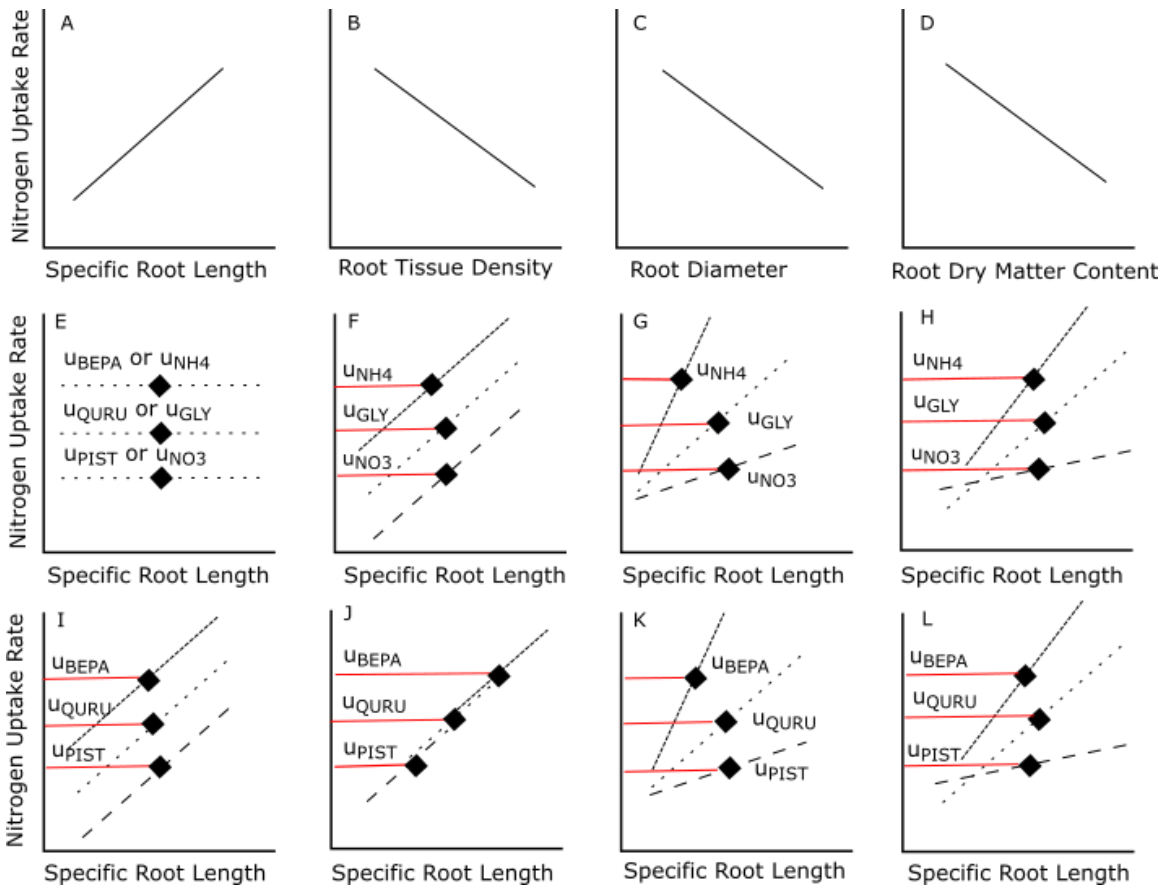

Figure 1.1. Hypotheses for the influence of root traits, species, and N forms on N uptake rate. A-D show proposed hypotheses for relationships between root morphological traits and N uptake, while E-L show mean uptake rates (u_x, diamond ◆) for different N forms and species. Solid (red) lines are drawn from the mean uptake rate (assumed to be at the mean value of specific root length) to show how different relationships between a morphological trait and N uptake rate (dashed lines (black)) could result in similar mean uptake rates for different N forms (E-H) or species (E, I-L). The species highlighted are ranked according to their leaf traits that are associated with an acquisitive resource use strategy, as we expected species with more acquisitive traits to have higher N demand and thus higher mean uptake rates.

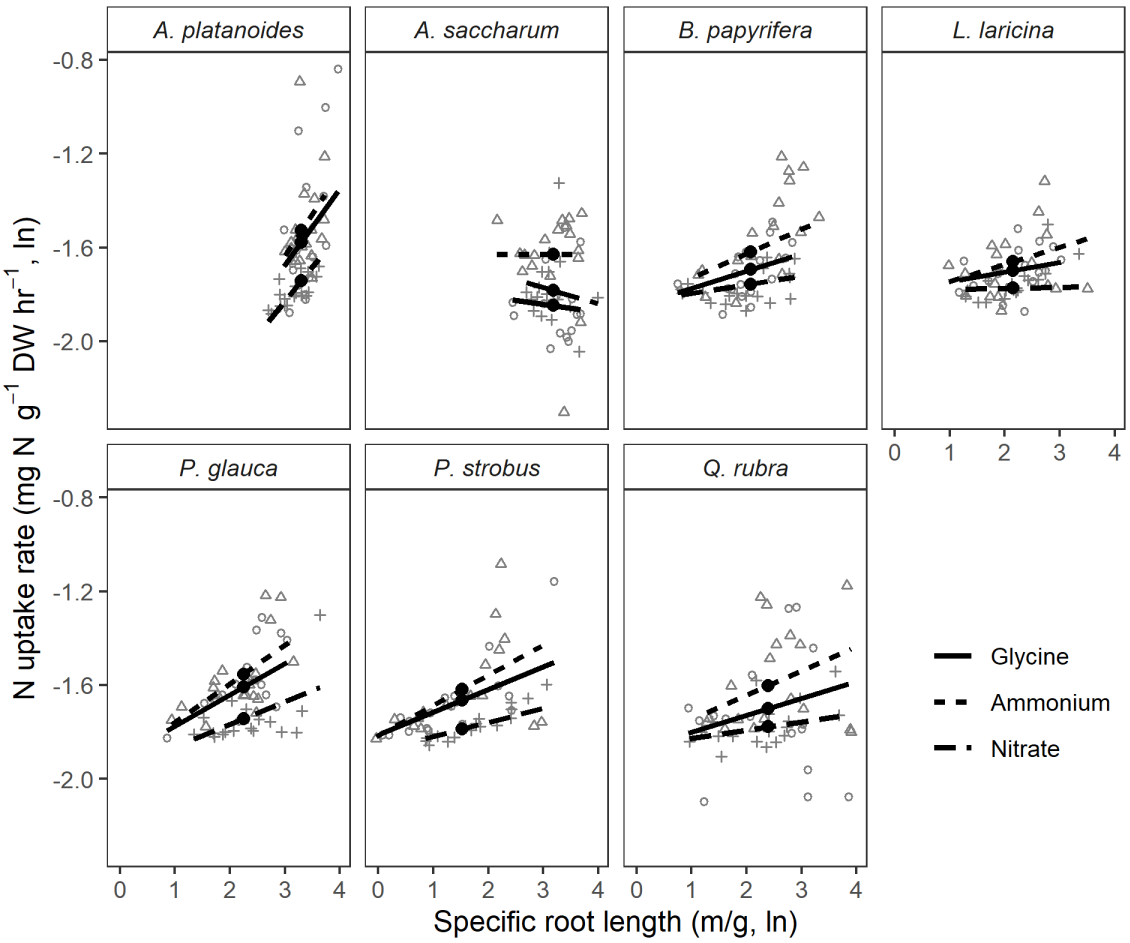

Figure 1.2. Nitrogen uptake rates show a positive relationship with specific root length. Solid circles indicate the mean uptake rate as estimated by the best fit GLS model at the species mean SRL. Both axes are on the natural log scale. All species except *A. saccharum* showed significant positive relationships of N uptake rate with SRL. SRL had the greatest impact on N uptake rate for NH_4^+ and for *A. platanoides* and *P. glauca*, as indicated by the estimates of their slopes. △ = uptake rates for ammonium, ○ = uptake rates for glycine, + = uptake rates for nitrate.

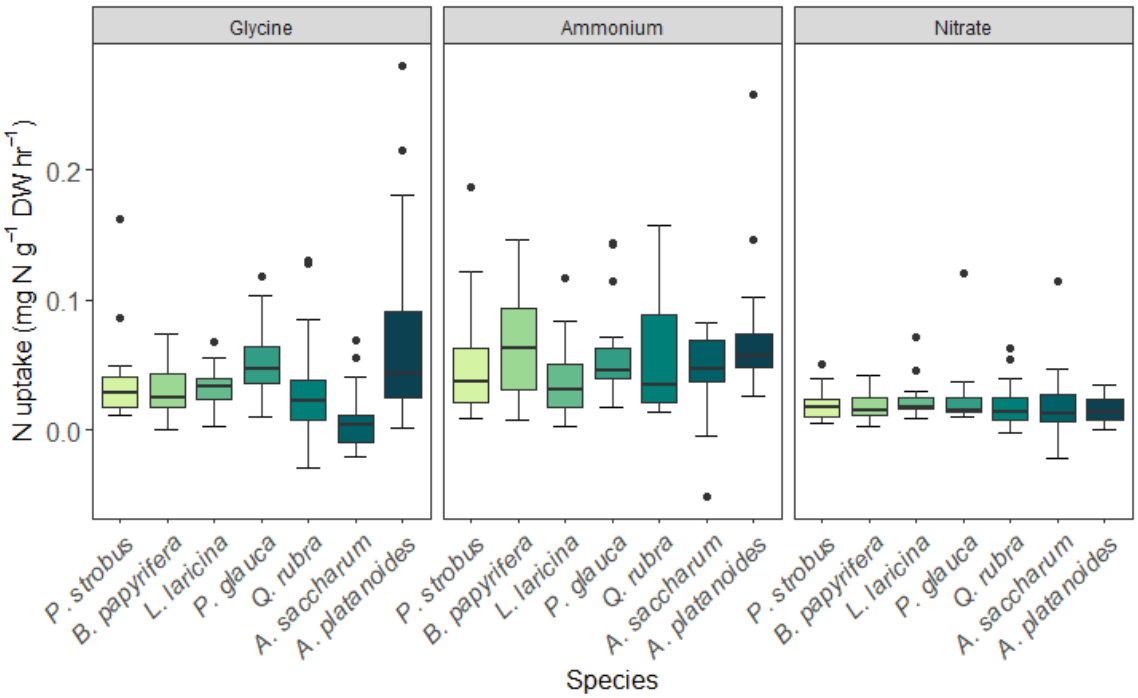

Figure 1.3. Nitrogen uptake rates vary by species and N form. Mean uptake rates for each N form and species combination along with the mean across all species; species are ordered from lowest to highest SRL values, corresponding to lighter to darker color. Uptake rates for all species were highest for NH_4^+ or $NH_4^+$and glycine. NO_3^- uptake rates were low across all species.

CHAPTER 2

Do plant traits help predict preferences for different nitrogen forms in tree species from the temperate-boreal forest ecotone?

Summary

Anthropogenic N deposition and climate warming can alter the predominant forms of nitrogen (N) available to plants. The impact of these changes could vary by species, as there is substantial evidence that plants differ in their capacity to take up and grow on different forms of N. However, predicting which species may be most affected by changes in N-forms remains challenging. This research examines whether plant traits associated with organic or inorganic nutrient economies and plant N demand are associated with preferences for a particular N form, indicated by an increase in growth on one form compared to an equal mixture of possible N forms. We hypothesized that species' preferences for different N forms will vary by their type of mycorrhizal association, latitudinal range, and N demand. To test this hypothesis, we grew seedlings of 11 temperate and boreal tree species in pots with different N-forms (amino acids, ammonium, nitrate, or an equal mixture of all N-forms). After one growing season, we measured total biomass, biomass allocation, leaf % N, and mycorrhizal colonization. The form of N supplied significantly affected plant growth across species, though not as hypothesized. NO_3^- was the N source preferred by all species regardless of latitudinal range or plant N demand (represented by leaf % N). Mycorrhizal type influenced the degree of preference for NO_3^-, as ectomycorrhizal species showed greater increases in

growth on NO_3^- than arbuscular mycorrhizal species. Overall, these results suggest that species in our study had similar capacity to use NO_3^- under the experimental conditions. Therefore, which species benefit from increases in NO_3^- could depend more on their ability to compete for this form, with species that are able to use more NO_3^- potentially having higher overall growth. These results help inform our understanding and guide future research on the impacts of changes in N cycling on species composition.

Introduction

Nitrogen is a limiting resource for plants in many ecosystems around the world (Du *et al.*, 2020), and competition for N plays an important role in regulating plant growth (Kulmatiski *et al.*, 2007) and structuring plant communities (Dybzinski & Tilman, 2007; Simon *et al.*, 2010). However, N availability is changing in many ecosystems worldwide as a consequence of both anthropogenic nitrogen deposition (Galloway *et al.*, 2014) and climate induced changes in N cycling within ecosystems (Dijkstra *et al.*, 2010; Butler *et al.*, 2012; Bai *et al.*, 2013). Both warming and anthropogenic N deposition can increase inorganic N availability (e.g. ammonium (NH_4^+) and nitrate (NO_3^-)) relative to organic N (Rennenberg *et al.*, 2009; Galloway *et al.*, 2014), altering the forms as well as the amount of N available to plants.

Changes in the availability of different N forms may have important consequences for plant community composition, as different species are likely to respond differently to increased N availability or changes in the forms of N available. However, while numerous studies have investigated how changes in total N availability impacts plant growth (Vadeboncoeur, 2010; Bassirirad *et al.*, 2015) and competition between species (Li *et al.*, 2015), studies that examine how changes in the forms of N that are available to plants impact these processes have produced variable results (Zerihun *et al.*, 1998; Britto & Kronzucker, 2013; Boczulak *et al.*, 2014; Rewald *et al.*, 2016). For example, many conifers have higher N uptake rates (Flaig & Mobr, 1992; Kronzucker *et al.*, 1997; Öhlund & Näsholm, 2004; Pritchard & Guy, 2005) and/or greater growth on NH_4^+ compared to NO_3^- (Öhlund & Näsholm, 2001; Metcalfe *et al.*, 2011), though this

preference is not universal (Zerihun *et al.*, 1998; Britto & Kronzucker, 2013). Other studies have shown greater uptake rates of organic N than either inorganic form (Scott & Rothstein, 2011), greater uptake rates of NO_3^- than of organic N or NH_4^+ (Boczulak *et al.*, 2014), or uptake proportional to each form's availability in the soil (Scott & Rothstein, 2011). Additionally, many studies examining the impacts of N form on plant N uptake and growth still omit organic N forms (e.g. (Nave *et al.*, 2013)), even though it is becoming increasingly clear that organic N forms are an important source of N for many species (Lipson & Näsholm, 2001; Hofmockel *et al.*, 2010).

The variability of species' responses in growth and N uptake, as well as the lack of research into species growth on organic N, make it difficult to predict the response of plant communities to changes in the forms of N available. These challenges may be particularly problematic for high-latitude ecosystems. While organic forms of N are widely available across a range of ecosystem types (Kielland *et al.*, 2007; Jones *et al.*, 2009; Hofmockel *et al.*, 2010; Rothstein, 2014), they may be more important in many colder ecosystems that have lower rates of net N mineralization (Lipson & Näsholm, 2001; Persson & Nasholm, 2001; Jones & Kielland, 2002; Schimel & Bennett, 2004; Näsholm *et al.*, 2009). Species may have adapted to better use organic N forms such as amino acids in these systems (Scott & Rothstein, 2011), and may be less poised to take advantage of increases in the availability of inorganic N forms like NO_3^-. At the transition zone between ecosystem types, such as the boreal-temperate forest ecotone, increases in inorganic N availability could thus favor temperate over boreal species. However, given the documented variability in N preferences there is less information about which species

may have greater growth or uptake of organic N compared to inorganic forms and which may be most impacted by changes in the relative availability of organic N to inorganic N.

One potential reason that it remains challenging to predict species responses to the availability of different N forms is that multiple characteristics can combine to influence how an individual species responds to the form of N available and rate of supply. Such characteristics include the type of mycorrhizal association, adaptation to different N supplies in soil (as captured by, e.g., geographic range), and plant economics spectrum traits. These characteristics capture information about both the availability of the different forms of N in a species' habitat as well as about plant N demand. For example, species that associate with arbuscular mycorrhizal fungi (AM) often inhabit soils with more inorganic nutrient availability compared to species that associate with ectomycorrhizal (EM) fungi (Phillips *et al.*, 2013). The fungi themselves can also differ in the types of N they are best able to acquire, as EM fungi can produce extracellular enzymes to help release N tied up in organic matter (Talbot *et al.*, 2013; Phillips *et al.*, 2014; Frey, 2019), and have been more widely shown to take up organic N than AM fungi (Talbot & Treseder, 2010). Similarly, a species' geographic range could also be tied to the availability of different N forms. Northern systems tend to be more N-limited (Pastor & Mladenoff, 1992; Franklin *et al.*, 2014), with lower rates of N cycling and lower inorganic N availability, in contrast to more southern systems, with warmer annual temperatures that can promote faster rates of N cycling and result in greater inorganic N availability (Reich *et al.*, 1997; Schimel & Bennett, 2004). Thus, species may sort along north-south latitudinal gradients according to their use of organic versus inorganic N forms, or have adapted to locally higher availability of different N forms. Species may

also have different temperature optima for the uptake of different forms based on their geographic range (Boczulak *et al.*, 2014), and so may differ in their N uptake rates for different forms in similar growing conditions. Finally, economics spectrum traits such as leaf N mass (Reich, 2014) indicate where a plant lies on the "fast-slow" traits continuum, whereby plants on the "fast" end of the PES, with high N requirements, may be adapted to quickly take up N in whichever form it is most available, and have weak preferences overall. In contrast, plants with "slow" strategies may not have the capability to respond quickly to changes in N form availability and be best suited to acquire one N form.

Here, we investigate whether species' preferences for different N forms can be predicted by their mycorrhizal association, latitudinal range, and/or plant economic traits (specifically leaf N concentration). We also examine whether any observed preferences depend on N availability. We use the term "preference" to reflect a plant species' relatively higher uptake and growth on a particular N form compared to other forms. We hypothesized that AM species and species from lower latitudes, typically associated with faster rates of N cycling, would show stronger preferences for inorganic forms of nitrogen (NH_4^+, NO_3^-) compared to EM species and species from higher latitudes, which may have weaker preferences or a preference for organic N. We also hypothesized that species with higher leaf N would show weaker preferences overall, as an adaptation to quickly use any N form available based on their higher N demand (Rothstein *et al.*, 2000). Finally, we hypothesized that species would show greater preferences with higher N availability. Differences in the cost of uptake or assimilation of each N form may become amplified at higher N levels as individuals are able to grow more overall, leading to greater variation in use of different N forms.

We tested these hypotheses using a suite of tree species common at the temperate forest-boreal forest ecotone. We selected eleven species that vary in their traits (mycorrhizal type, geographic range, and leaf %N) and grew them from seed in a greenhouse in eight different treatments in which we varied N form and N levels. We measured their growth and biomass allocation to roots versus shoots. Preferences for different N forms were determined by comparing differences in seedling biomass between seedlings grown on one form (amino acids, NH_4^+, NO_3^-) to those grown on an equal mixture of organic and inorganic N forms. We then looked for relationships between these preferences and species' mycorrhizal type, geographic range, and leaf N to test whether the species and traits we had selected produced predictable patterns in the form preferred or the overall strength of their preferences.

Materials and Methods

Plant material

The 11 species used in this study (Table 2.1) were selected based on their presence at the temperate-boreal forest ecotone in Minnesota and to span a range of plant traits that may impact plant N preferences. We aimed to have representation of temperate and boreal species, and both ectomycorrhizal and arbuscular mycorrhizal species that cover a wide range on the leaf economic spectrum (indicated by their mean leaf N content). These traits are also summarized in Table 2.1. Species included three deciduous broadleaf (*Acer saccharinum*, *Acer rubrum*, *B. papyrifera*) and one deciduous conifer species (*L. laricina*), in addition to 7 evergreen conifer species (*Abies balsamea*, *Picea*

glauca, *Picea mariana*, *Pinus resinosa*, *Pinus strobus*, *Pinus banksiana*, and *Thuja occidentalis*). Seeds for all species except *T. occidentalis* were collected in Minnesota in the same area to minimize the chance that local adaptation to different environmental conditions would influence their responses to the different nitrogen treatments (Boczulak *et al.*, 2014). Seeds for the maples were collected by the Minnesota Department of Natural Resources in late May-Early June 2016 in Northern Pine County, MN or the Duluth, MN area, while seeds of the other species were collected between 2008 and 2013 and had been cold stratified prior to planting. *T. occidentalis* seeds were ordered from Sheffield's Seed Company (www.sheffields.com) and were collected in Michigan.

Growth conditions

Seeds were soaked in deionized water for 24 hours prior to planting to facilitate germination. Five to fifteen seeds were sown in plastic conetainers (D40 Deepots, Steuwe and Sons, Inc., Tangent, OR, USA) depending on the average germination percent of a species and thinned to one individual per pot for species with larger seedlings (e.g. *Acer saccharinum*) or three for smaller/slower growing species (e.g. *Picea mariana*). Seedlings were grown in a mix of field soil and sterile sand/soil mixture (Ratios: 10% field soil, 22.5% sterile soil, 67.5% sand). Sand was the predominant component to minimize additional nutrients from the growth media. A small amount of field soil was used to provide an inoculum from a natural soil community to facilitate the formation of mycorrhizal associations in the tree seedlings (Bruns *et al.*, 2009). Field soil was collected from the top 30cm of soil after removing the litter layer in natural stands with

loamy sand soils dominated by *B. papyrifera*, *P. strobus*, *P. resinosa*, and *A. balsamea* at the Cloquet Forestry Center, Cloquet, MN in June 2016.

Seedlings were grown in a temperature-controlled greenhouse (20 °C/25 °C) at the University of Minnesota, St. Paul for *ca* 100 days starting in June 2016, with 15h daylength (supplemented by greenhouse lights starting August 2016). Maples were harvested earlier (after *ca.* 80 days) to avoid leaf senescence but had grown substantially by that time. Seedlings were watered daily or every other day with deionized water depending on soil conditions. To avoid any impacts of a seedling's location (in the greenhouse or in an individual tray) on seedling growth by species or treatment, each species was randomly assigned a location within a tray and trays were grouped by treatments in blocks. Each block was rotated throughout the greenhouse every other week.

Nutrient treatments

Nutrient solutions were added to pots two times per week throughout the growth period. Nitrogen treatments consisted of two N levels crossed by four N forms for a total of 8 treatments. One additional no-N treatment was also included as a control for any residual N from the field soil (Table 2.2). The two N levels were selected to bracket the normal range of N availability in the ecotone (SE Hobbie, unpublished data): a low N treatment of 0.5 mg N wk^{-1} and a high N treatment of 2 mg N wk^{-1}. For each N level, N was applied as one of four different N form treatments: organic N (supplied as a mixture of three amino acids: glycine, arginine, and glutamine); ammonium supplied as

ammonium sulfate ($(NH_4)_2SO_4$); and nitrate supplied as calcium nitrate ($Ca(NO_3)_2$). Additional nutrients were supplied following a traditional nutrient solution used in conifer nurseries (Öhlund & Näsholm, 2001): P (1 mM, 90 mg L^{-1}); K (1.5 mM, 170 mg L^{-1}); Ca (5.4 mM), Mg (3.1 mM), S (2.5 mM), and micronutrients (Na (0.4 mM), Fe (12 µM), Mn (4.7 µM), B (2.4 µM), Cu (0.3 µM), Zn (0.7 µM), Mo (0.07 µM)). The pH of each solution was checked with pH indicator strips (CN: 2613-991, Whatman Co., Germany) before application to confirm that pH remained between 5-6, which is typical of soils in this ecotone (Alban, 1982). Nutrient solutions were always applied after watering for the day to avoid flushing nutrients before plants could take them up.

Measurements, harvesting, and biomass processing

The germination date of seeds in each pot was recorded daily for the first two weeks and weekly thereafter. Height of each seedling was measured every other week from the base to the top of the stem. As some individuals died during the experiment, the date of death was recorded for each individual. At the end of the growth period, seedlings were harvested by gently sliding the individuals out of the pot and rinsing the roots with DI water to remove soil particles, and the total length of the root system was measured. Seedlings were separated into above and belowground biomass before drying in an oven at 65 °C for 48 hrs. Prior to drying belowground biomass, the entire root systems of EM species were visually scored for mycorrhizal colonization on root tips to calculate the percent colonization (Kubisch *et al.*, 2015). Total dry mass for each pot was determined by summing the mass of the above- and belowground biomass, and for pots with more

than one seedling we divided by the number of seedlings to obtain an average biomass that was used in remaining calculations. We measured above and belowground biomass separately so we could determine whether species differed in how they allocated their biomass. Differences in investment to below- versus above-ground biomass could show evidence of N limitation that might be hidden from just examining total biomass (Grier *et al.*, 1984) and can influence species' performance in future growing seasons (Gruffman *et al.*, 2012).

Total leaf N was determined for two species with the greatest survival to determine whether growth patterns matched N acquisition patterns. Leaf tissue from *A. saccharinum* and *P. banksiana* was dried at 60 °C, ground and weighted into tin capsules before analyzing the C and N content on a Costech ECS Element Analyzer (Model 4010) at the University of Minnesota.

Calculations and statistical analyses

The latitudinal range of each species was determined using data from the Forest Inventory and Analysis database for Minnesota (https://www.fia.fs.fed.us/tools-data/index.php). For each species, the percent abundance north of the temperate-boreal forest ecotone was determined by dividing the total abundance of each species in the three counties on the north end of the ecotone (Cook, Lake, St. Louis) by the total abundance of each species in the ecotone (which included the additional counties of Aitkin, Crow Wing, and Carlton).

To enable comparison of N preferences across species we needed to account for significant differences in total mass by species. We did this by calculating the response ratio of biomass when grown on each N form individually relative to the mixture treatment at the corresponding N level (Eq 1).

$$\textbf{Eq. 1}: Biomass\ Response\ Ratio\ (BRR) = \ln \frac{Biomass_{trt}}{Biomass_{mix}}$$

All statistical analyses and calculations were conducted in R version 4.0.0 (R Core Team, 2020). Before analysis, we checked for outliers in the data using the *outliers* package (v. 0.14) and found variables that contained values greater than 99% of others using the chi-square test. We then examined Cleveland Dot Plots for those variables to see if there were extreme high or low values that were likely to influence our analyses (Zuur *et al.*, 2007), and did not find any outliers using this method.

We first compared whether species differed in total biomass using linear models with species, N form, and N level as predictors as well as all potential interactions. We used backwards model selection using the *stepAIC* function (*MASS* package v. 7.3-51.5) to find the best model and then used the *Anova* function (*car* package v. 3.0-7) to run a type III SS analysis of variance to test whether N form or N level influenced growth by species. We followed up with pairwise comparisons for significant predictors using the *emmeans* function (*emmeans* package v. 1.4.6) with the Tukey adjustment for multiple tests. In this analysis, we excluded 4 species (*A. balsamea*, *L. laricina*, *P. glauca*, and *P. mariana*) that had low replication due to high mortality before harvest and instead compared survival by N form and N level using a binary logistic regression (*glm* function, *stats* v. 4.0.0). Since individuals may not allocate biomass to roots vs. leaves in

a 1:1 ratio as they grow, examining just the root mass fraction (root mass over total mass) may result in species differences simply due to differences in the size of individuals even if they have similar allocation relationships. So, we also tested for growth differences in biomass allocation by species and N treatments using standard major axis (SMA) regression (Warton *et al.*, 2012). We examined differences in the allometric relationship between leaf and root mass by species and N form (indicated by differences in slope), as well as total differences in allocation to roots versus leaves (indicated by differences in elevation/intercept) (Warton *et al.*, 2012). We also used this to look for differences in allocation by N form, N level, and mycorrhizal type.

To test our primary hypotheses, we looked at differences in the biomass response ratio (BRR) by N form and N level with plant traits. We fit linear models with BRR as the response variable and N form, N level, and each trait (leaf N, geographic range, mycorrhizal type) as the predictors. We first fit models with all potential interactions and then followed the same protocol described for comparing total biomass by species for finding and summarizing the best fit model. We tested for the influence of N level on patterns in N preferences by species or by trait by checking for interactions between N form and N level in our previous analyses. We conducted additional ANOVAs on supplemental data (leaf N content in *A. saccharum* and *P. banksiana*; percent colonization of roots by EM fungi on *P. strobus* and *P. banksiana*) to see if these varied by N form and N level and could help explain any patterns in biomass. For all analyses, we used the *check_model* function (*performance* package v. 0.4.7) to visually confirm that our data did not violate model assumptions.

Results

Do species traits predict preferences for different N forms?

None of the traits examined explained much variation in seedling growth responses to different N forms. For all traits, models with each individual trait plus N form and N level had AIC and BIC values that were slightly (Range, Leaf N) or substantially higher (mycorrhizal type, MF) than the baseline model with just N form and N level (Table 2.3). In the case of leaf N and range limit, while both variables were retained in the best fit model (Table 2.4), neither trait increased the variation explained by the model with just N form and N level. There was a slight increase in the BRR with species range at high N levels, but this was highly variable and only for NO_3^- (Fig. 2.1). Leaf N had no trend with BRR (Fig. 2.2), though the BRR was still higher for NO_3^-. Similarly, though MF type was retained in the best fit model, it did not increase explanatory power compared to the baseline model given its higher AIC and BIC values and lower R^2. However, there were some trends in the response by mycorrhizal type: at high N levels, ECM species had slightly higher increases in biomass than AM species for the amino acid and NO_3^- treatments, and tended to have lower biomass on the ammonium treatments than the AM species ($t = -1.76$, $p = 0.08$, Fig. 2.3).

Seedling growth and nitrogen content differed by species, nitrogen treatment and nitrogen level

Since plant traits explained little variation in seedling biomass response, we also examined the responses of individual species to determine if any species differed in their

growth on each N form. However, species did not differ substantially in their response to the N form treatments. The Species * N form interaction was dropped from the best fit model (Table 2.5), so even though there was substantial variation in total plant biomass by species (24 of 30 pairwise comparisons significant at $p < 0.01$), with *A. saccharinum* accumulating *ca*. 80X more biomass than *P. glauca*, species responded similarly to the N form treatments (Fig. S2.1). While neither N level nor N form were significant predictors of plant biomass alone, their interaction remained in the best model for total biomass (Table 2.5). At low N levels, seedlings within a species grew comparably on all N forms ($p > 0.35$ for all), while at high N levels the seedlings grown on the mixture and NH_4^+ treatments were only ~60% and 70% the size of those grown on the NO_3^- treatments, respectively ($t = -3.53$, $p = 0.003$, $df = 182$; $t = -2.35$, $p = 0.091$, $df = 182$; Fig. S2.1). There was also variation in species' responses to the N level treatment, with some species growing much more at higher N levels (e.g. *B. papyrifera*, L:H = 0.295, $p < 0.0001$), while others had similar biomass at low and high N (e.g. *P. banksiana*, L:H = 0.886, $p = 0.46$).

We measured leaf N content for two species to evaluate whether differences in total biomass by treatment corresponded to differences in N acquisition. The two species we measured for final leaf N content at the completion of the growth period differed in their leaf N levels as well as the relationship between leaf N and plant biomass (Tables 2.6, 2.7). *A. saccharinum* had about 10% higher leaf %N compared to *P. banksiana* ($t = 2.08$, $p = 0.04$), while both species had about 20% higher leaf %N on the high N versus the low N treatment ($t = 4.36$, $p < 0.0001$ Fig. S2.2). Additionally, in *A. saccharinum*, leaf %N was strongly and negatively correlated with both leaf biomass and whole plant

biomass across all N treatments except the high-N mixture and NO_3^- treatments (Table 2.7). In contrast, *P. banksiana* varied in the strength and direction of the correlations, and only the low-N amino acid treatment showed a strong positive correlation between leaf %N and plant biomass ($r = 0.96$, $p = 0.04$ for leaf biomass). In contrast to leaf %N, total N accumulation in leaves did vary by N form treatment (Table 2.8). The best-fit model for total leaf N included a significant main effect for N form and N level as well as an interaction between N form and N level, species and N level (Table 2.8). *A. saccharinum* had higher total N in leaves than *P. banksiana*, and this difference was greater at the high N level (Fig. 2.2, $p < 0.0001$ for both). However, both species had similar responses to the N form treatments: at the low N level, neither species had large differences in total leaf N by N form, but at high N levels the mixture N form treatment had the lowest total N compared to the other three N forms (AA: $t = 3.26$, $p = 0.009$; NH4: $t = -2.85$, $p = 0.029$; NO3: $t = -3.86$, $p = 0.001$).

Biomass allocation differed by species and N level but not by N treatment

We did not find any variation in biomass allocation to roots versus leaves by N form but did see differences in allocation by N level and species. When comparing allocation by N form across species and N levels, allocation to roots versus leaves was similar for all forms both in terms of the slope of the relationship (Log likelihood (LL) ratio = 0.128, $p = 0.99$) and intercept (Wald = 0.542, $p = 0.91$). However, allocation to roots did increase at the low N level as seen by a significant difference in the intercept between the high and low N levels (intercept -0.64 vs. -0.58 in L vs H, $p = 0.017$), though

the slope of that relationship did not vary by N level. When comparing allocation by species, species primarily varied in the amount of biomass allocated to roots compared to leaves (Wald = 174.2, $p < 0.0001$) though there were small differences in how allocation changed with size for some species (LL ratio = 26.4, $p = 0.003$). Notably, in *A. saccharinum* and *A. balsamea*, allocation to roots was greater than for other species with similar biomass (Fig. 2.5A). We also compared allocation by MF type to see if they responded differently to the N form treatments. However, while the relationship of allocation to roots versus leaves did vary by MF type (Fig. 2.5B), within MF types allocation did not vary by N form. For AM species, allocation to roots increased more than allocation to leaves in larger individuals compared to EM species, as evidenced by the shallower slope for AM versus EM species.

Colonization of root tips by ECM fungi varies by species, N form, and N level

Colonization of root tips by ECM fungi was low overall for both *P. banksiana* and *P. strobus*, and the patterns of colonization by N form and N level varied by species. For *P. banksiana*, percent colonization was higher on the low N treatment versus the high N treatment, but this varied by form (Table 2.9). Only the low mix treatment differed from the high mix and high amino acid treatments in percent colonization ($t = 3.21$, $p = 0.04$ and $t = 3.08$, $p = 0.059$, respectively), while the high-N ammonium and nitrate treatments had similar colonization to the other low-N treatments (Fig. S2.2). Similarly, the effect of N level on colonization for *P. strobus* depended on the N form, though there was less variation in percent colonization overall: only the high N amino acid and high N

ammonium treatments differed in their colonization, with about 3X greater colonization on the amino acid treatment compared to the ammonium treatment ($t = 3.23$, $p = 0.04$). We did not include the no-N treatment in the analysis, but colonization of root tips was either similar to the low N treatment (*P. banksiana*) or higher than the low N treatment (*P. strobus*). Colonization of roots for other ECM species were either low enough that they were hard to visualize (*P. resinosa* and *P. glauca*), or the species had enough mortality (*A. balsamea* and *P. mariana*) to make comparisons by N form and N level difficult.

Survival of species varied by N level but not N form

Survival of seedlings varied by N level and species but not by N form. For example, *A. saccharinum*, *A. rubrum*, *T. occidentalis* had extremely high survival compared to *Picea mariana*. For the former three species, survival was at or near 100%, while for *P. mariana* survival was much lower. For the three species with the highest mortality (*B. papyrifera*, *P. mariana*, and *P. resinosa*), we examined whether survival was impacted by the N treatments. Survival for these species varied by N level, as individuals in the high N treatments had lower odds of dying compared to the low N treatment (Odds ratio = 0.37, z-value = -2.00, $p = 0.046$). Survival did not vary by N form, as indicated by the larger AIC and BIC values for the model with N form compared to the model without it, but did vary by species (Table S2.1). *P. mariana* had greater odds of dying compared to both *B. papyrifera* (z-ratio = -2.72, $p = 0.018$) and *P. resinosa* (z-ratio = 2.06, $p = 0.098$).

Discussion

Our experiment tested whether plant traits could help predict preferences for different N forms across eleven tree species. We had expected to see variation in preferences for different N forms, specifically that species from higher latitudes, with EM fungal associates, and with lower leaf N content would show greater growth on amino acids or ammonium compared to nitrate or the mixture treatments. Yet all species that exhibited a preference for an N form preferred nitrate, as indicated by greater growth on nitrate at high N levels compared to the other N forms supplied. In the two species on which we measured final N content (*P. banksiana* and *A. saccharinum*), greater N uptake accompanied greater growth on nitrate. While we expected that AM species and species from lower latitudes would prefer nitrate, many of the conifers showed greater growth on nitrate, which we did not expect, and which was counter to some previous studies that emphasized low nitrate uptake by conifers (Kronzucker *et al.*, 1997; Gessler *et al.*, 1998; Malagoli *et al.*, 2000). Additionally, some species in our study did not show preferences for one N form at all (e.g. *A. rubrum*, *P. glauca*, *P. resinosa*), even at high N levels, which we had expected to accentuate differences in preferences for N forms. Other studies also have shown that not all species exhibit clear preferences for different N forms (Warren & Adams, 2007; Gloser *et al.*, 2008).

Differences in uptake and assimilation help explain preferences for nitrate

Though the almost universal preference for nitrate in our study was not anticipated, there are several reasons that could explain greater growth on nitrate for most

species. First, many past studies that showed preferences for ammonium over nitrate in some species measured N uptake rates rather than overall growth (Jackson & Reynolds, 1996; Schulz *et al.*, 2011; Liu *et al.*, 2017). While uptake rates may be important in explaining N preferences of species growing in competition (Simon *et al.*, 2010, 2014; Li *et al.*, 2015), a species with low uptake rates for nitrate may still have been able to access adequate nitrogen resources in the absence of competition. Second, one of the reasons we hypothesized that some species would prefer ammonium over nitrate was that nitrate must be reduced to ammonium before it can be incorporated into plant biomass, which adds an additional cost to nitrate assimilation (Miller & Cramer, 2004; Britto & Kronzucker, 2013). However, continuous growth on nitrate could have induced nitrate reductase activity (NRA) in leaves, even in some of the conifers (Smirnoff *et al.*, 1984; Smirnoff & Stewart, 1985; Andrews, 1986; Downs *et al.*, 1993; Min *et al.*, 1998). Greater NRA in leaves could be associated with lower assimilation costs through coupling with photosynthesis/photorespiration (Bloom, 2015a), making it a more favorable form to take up. Additionally, ammonium must be assimilated into plant biomass quickly as it can be toxic to plants in large quantities (Britto & Kronzucker, 2002), while nitrate can be stored in plant tissues before assimilation, potentially favoring plant growth on nitrate over ammonium. As the ammonium treatment tended to support the lowest growth of the single N form treatments, especially in *P. strobus* and *B. papyrifera*, this toxicity could have been one reason for the reduced growth seen in our experiment. Finally, nitrate uptake has been found to peak at higher temperatures than either ammonium or amino acid uptake (Vaast *et al.*, 1998; Boczulak *et al.*, 2014). While the greenhouse was temperature controlled, it was likely still warmer than field soil conditions for many

species (Gessler *et al.*, 1998), and thus could have promoted higher nitrate use than expected based on previous studies.

Many species grew well on the amino acid treatment, while the mixture treatment tended to have the lowest biomass or lowest N content. While evidence is growing that organic N forms are an important N source for many plant species (Lipson & Näsholm, 2001; Näsholm *et al.*, 2009), we did not expect that AM species would show greater growth on amino acids than the mixture treatment because they tend to be associated with soils with greater inorganic nutrient availability (Phillips *et al.*, 2013). Yet, one of the AM species (*A. saccharinum*) grew ~30% more on this treatment compared to the mixture and ~12% more than on the ammonium treatment. Root transporters for amino acids are thought to be very widespread (Miller & Cramer, 2004; Rentsch *et al.*, 2007), and assimilation costs may be less for amino acids since they are already attached to a carbon skeleton (Franklin *et al.*, 2017). It is also possible that soil microorganisms converted amino acids into ammonium and/or nitrate, potentially explaining the unexpected growth of species like *A. saccharinum* on this treatment. We cannot eliminate the possibility that this occurred; however, there are differences in growth patterns across the N form treatments that suggest any transformations did not eliminate the effect of supplying species with different N forms. For example, *A. rubrum* had reduced growth on amino acids compared to the mixture treatment, in contrast to species like *B. papyrifera* that had greater growth on amino acids. If transformations had made each N form treatment similar, we would not have expected to see such noticeable differences in growth on the different N forms relative to the mixture.

Given that the species in our study all seem to have the capacity to use each N form, we were initially surprised that the mixture treatments had the lowest growth. However, since each N form requires its own active transporter for uptake into the root (Vidmar *et al.*, 2000; Miller & Cramer, 2004; López-Pedrosa *et al.*, 2006; Rentsch *et al.*, 2007), it may be costly to maintain transporters for all forms. The added cost of maintaining multiple transporters could have resulted in reduced growth on the mixture relative to the single source treatments seen in most species. It may therefore be a more effective strategy for plants to only transcribe one type of transporter to acquire N, though this warrants further investigation. Another potential reason for the reduced growth on the mixture is that ammonium can interfere with the uptake of nitrate (Gessler *et al.*, 1998; Vaast *et al.*, 1998), which may have effectively reduced the amount of N available to plants on the mixture treatment. While reduced growth and leaf N in the mixture treatment were primarily seen at high N, this could be because species have different types of transporters that they use at different N concentrations. Ones used at low concentrations, high-affinity transport systems (HATS), tend to have lower maximum uptake rates than those used at higher concentrations, low-affinity transport systems (LATS) (von Wirén *et al.*, 2000; Miller & Cramer, 2004). If concentrations of each form in the mixture were not high enough to trigger transcription of the LATS for each form, uptake capacity of species in the mixture treatment could have been reduced on high N more so than on low N.

Importance of N availability

We observed the expected trends in the effects of N level on seedling preferences for different N forms, with stronger preferences observed at high N levels compared to low N levels. Though this is what we expected, we could also have seen that extra costs of assimilating one form over another were more pronounced at lower N levels. However, it is possible our low N levels were low enough that N limitation outweighed any differences in uptake or assimilation by form. While we used N levels that should have bracketed the range of N availability in the field, the generally lower growth, lower leaf N, and slightly higher allocation to roots versus leaves on the low N level suggests that seedlings were N limited. Additionally, it is possible that the transporters used at low N levels are more similar in their uptake rates (Kiba & Krapp, 2016), and so forms are less differentiated at low N levels. Furthermore, transporters for each form can be present at low external N concentrations even for nitrate (Zhang *et al.*, 2018). The presence of these transporters may represent a sunk carbon cost for plants, so that at low N seedlings may have similar costs for taking up each form and the low N is the stronger growth-limiting factor.

Why were traits unimportant in explaining N preferences?

Several reasons could explain why ECM-associated species preferred nitrate over other N forms, in contrast to our expectations. First, the stronger response of ECM species to nitrate compared to AM species could be explained by differences in allocation to roots versus shoots between the species groups. ECM species had less allocation to

roots than AM species, which indicates that ECM species could exploit less soil volume for N (Leffler *et al.*, 2013). Since nitrate is more mobile in most soils than the other forms (Miller & Cramer, 2004), it could potentially diffuse more readily to ECM roots making more N accessible to them than on the other forms. Second, low allocation to roots may have been compounded by low colonization of roots by ECM fungi, which typically help them explore more soil volume (Hawkins *et al.*, 2015). While we supplied the seedlings with inoculum via field soil, there were no established trees in our pots to provide additional inoculation through vegetative means, which can promote rapid colonization of seedlings in the field (Fleming, 1984). Third, the fungal taxa colonizing the roots of these seedlings might also have favored nitrate uptake. ECM fungi are a diverse group of organisms that vary widely in their enzymatic capacity and growth forms (Agerer, 2001; Smith & Read, 2008; Hobbie & Agerer, 2010), and while many can help plants access organic forms of N, there are some more ruderal, faster growing ECM fungal species that may be better at taking up nitrate (Gobert & Plassard, 2002; Avolio *et al.*, 2009). We were not able to identify the species colonizing the roots of the fungi, but given the short time frame of our study, ECM fungi that colonized roots in this study may have been these more ruderal taxa that would also facilitate nitrate uptake over the other N forms.

That species with more northerly range limits preferred nitrate, in contrast to our expectations, might be explained by additional traits of the northern species in the study that influenced N uptake in ways that obscured any potential correlation with range limits. For example, two of the more northern species are relatively shade intolerant (*B. papyrifera*, *P. banksiana*) (Perala & Alm, 1990 and Burns & Honkala, 1990, in Reich *et al.*, 1998), which could be related to the potential for nitrate uptake as NRA in leaves is

light-dependent and for some species can occur at higher rates in leaves versus roots (Downs *et al.*, 1993; Thomas & Hilker, 2000). Additionally, these species tend to recruit after disturbance when inorganic N availability may increase (Turner *et al.*, 2007; Kong *et al.*, 2015), so seedlings of these species may be better adapted to take up and use nitrate compared with other northern species. Two of the more shade-tolerant, boreal species (*A. balsamea, P. mariana*) had high mortality, which made comparisons across forms difficult, though we did note that survival of these species did not vary by N form.

A preference for amino acids by species characterized by low leaf N might have been obscured by slow growth in those species, making it hard to detect differences in biomass. However, we do not think this is the main reason we did not see preferences for those forms, as one of the slower growing species with low leaf N, *T. occidentalis*, showed a trend of increased growth on NO_3^- compared to the mix treatment. Additionally, we had expected that some faster growing species would show weaker preferences, yet the two species with the highest leaf N had greater growth on NO_3^- as well. It is therefore possible that leaf N and associated traits on the plant economics spectrum do not explain variation in preferences for certain N forms in isolation; rather, these traits may be more important for explaining competition for N or rates of N uptake rather than variation in acquisition of different N forms.

Implications and other considerations

While there are important points to be taken from our work, it is equally important to acknowledge the ways in which our results may or may not be applicable to

seedling growth and preferences in the field. In addition to the previously mentioned reasons that NO_3^- may have been favored in our study, there are other factors that could favor different forms in the field we have not touched on. For example, soil pH has been shown to impact uptake of different N forms, with higher pH tending to favor nitrate uptake over ammonium (Meng *et al.*, 2016). While our nutrient solutions were between pH 5-6, field soil pH can be lower (~4.5) under conifers (Alban, 1982). Greater NO_3^- uptake has also been linked to soils with higher % base saturation and especially calcium content (D'Amore *et al.*, 2009), so since we supplied our plants with these necessary nutrients they could be more applicable to soils with those properties. Since conifers tend to occur on more acidic soils (Augusto *et al.*, 2015), even if they may grow better on NO_3^-, field conditions may hinder the uptake nitrate. Acidic and conifer-associated soils also have been shown to have low nitrification rates and low NO_3^- concentrations (Venterea *et al.*, 2003), so their capacity for growth on NO_3^- may not be important in most field conditions. Additionally, field soils may exhibit more fluctuating resource levels rather than a constant supply of amino acids, NH_4^+, or NO_3^- (Laverman *et al.*, 2000). Fluctuating resource levels could favor a more balanced uptake strategy. As mentioned previously, field soils may also be cooler than the conditions in the greenhouse. Conducting the experiment with cooler soil temperatures could have increased survival of some of the boreal or more shade-tolerant species as well as potentially reduce NO_3^- uptake, and may have resulted in some species showing increased growth on amino acids or NH_4^+ rather than NO_3^-. Finally, soil moisture can influence which N form is best for plant growth. Organic N forms have been shown to increase plant growth compared to NH_4^+ and NO_3^- under drought conditions (Sigala *et al.*,

2020), so drier field conditions could favor species using organic N. Despite these considerations, the work presented here provides important additional information on how different N resources are used by trees and the conditions under which certain forms may enhance the growth of some species over others.

Conclusions

Our work provides further evidence that the chemical form of N available to plants impacts their growth. In our greenhouse study, nitrate was the form that resulted in the highest growth in most species, though we highlight external conditions that could favor use of other N forms under field conditions. Though we did not observe clear patterns of N form preference based on species traits, we highlight potential additional traits that may have contributed to plant N form preferences and obscured the expected relationships. Determining whether there are traits associated with N form preferences remains important for understanding how changes in N forms available to seedlings could favor certain species over others in the future, since changes in the forms of N available to plants are likely due to various global change factors (Bai *et al.*, 2013; Galloway *et al.*, 2014).

Table 2.1. Study species and relevant characteristics. Range classifications pulled from USFS Fire Effects Information System*; % North (N) of Ecotone calculated from FIA data (see methods); Leaf % N (per unit mass) from the GLOPnet database (Wright *et al.* 2004). In the Range column, an (S) indicates species with more southern ranges within the boreal category.

Species	**Family**	**MF Type**	**Range**	**% N of ecotone**	**Leaf % N**
Abies balsamea	Pinaceae	EM	boreal	87.2	1.66
Acer saccharinum	Sapindaceae	AM	temperate	9.2	2.52
Acer rubrum	Sapindaceae	AM	temperate	54.6	1.91
Betula papyrifera	Betulaceae	EM	boreal	82.3	2.31
Larix laricina	Pinaceae	EM	boreal	62	1.36
Picea glauca	Pinaceae	EM	boreal	92.3	1.28
Picea mariana	Pinaceae	EM	boreal	93.2	1.02
Pinus banksiana	Pinaceae	EM	boreal (S)	94.4	1.10
Pinus resinosa	Pinaceae	EM	temperate	78.4	1.07
Pinus strobus	Pinaceae	EM	temperate	86.7	1.42
Thuja occidentalis	Cupressaceae	AM	boreal (S)	94.9	0.99

*Species review section: https://www.feis-crs.org/feis/
Abbreviations: MF type = mycorrhizal type; EM = ectomycorrhizal; AM = arbuscular mycorrhizal

Table 2.2. Composition of nitrogen treatments and their concentrations. N levels were calculated to bracket the typical range of N availability found in this ecotone (S. Hobbie, unpublished).

N Level	N Treatment	Amino acids	NH_4^+	NO_3^-
Low	Amino acids	90 mg L^{-1}	5 mg L^{-1}	5 mg L^{-1}
	NH4	5 mg L^{-1}	90 mg L^{-1}	5 mg L^{-1}
	NO3	5 mg L^{-1}	5 mg L^{-1}	90 mg L^{-1}
	Mixture	33.3 mg L^{-1}	33.3 mg L^{-1}	33.3 mg L^{-1}
High	Amino acids	360 mg L^{-1}	20 mg L^{-1}	20 mg L^{-1}
	NH4	20 mg L^{-1}	360 mg L^{-1}	20 mg L^{-1}
	NO3	20 mg L^{-1}	20 mg L^{-1}	360 mg L^{-1}
	Mixture	133.3 mg L^{-1}	133.3 mg L^{-1}	133.3 mg L^{-1}
No-N	Macro- and micronutrients	0 mg L^{-1}	0 mg L^{-1}	0 mg L^{-1}

Table 2.3. Performance of models explaining variation in biomass response ratios with different traits. Models were compared to the baseline model with just N form treatment to test whether including the traits improved model fit.

Model (Subset)	**AIC**	**BIC**	**R^2-Adj**	**RMSE**
N Form + N Level	240.9	256.0	0.13	0.52
+ Range + Range:N Level	241.3	262.4	0.14	0.52
+ Leaf N + Leaf N:N Level	242.8	263.9	0.13	0.52
+ MF	253.7	268.7	0.05	0.54

Table 2.4. ANOVA table for models of the biomass response ratio with individual traits.

Trait	Predictors	F-value	p-value	df
% Range North of Ecotone	Intercept	1.04	0.31	1
	N Form	3.94	0.02	2
	N Level	0.14	0.71	1
	Range	0.75	0.39	1
	Range:N Level	3.3	0.071	1
Leaf N	Intercept	5.57	0.02	1
	N Form	3.91	0.02	2
	Leaf N mass	1.43	0.23	1
	N Level	7.16	0.008	1
	Leaf N mass:N Level	1.95	0.16	1
MF	Intercept	2.48	0.12	1
	N Treatment	3.48	0.033	2
	MF	3.1	0.08	1

Table 2.5. ANOVA table for the best model comparing seedling biomass by species, N form, and N level.

Predictor	Sum Sq	Df	F value	p-value
Intercept	3.63	1	77.3	< 0.0001
Species	34.8	5	148.5	< 0.0001
N Level	0.02	1	0.41	0.52
N Form	0.19	3	1.33	0.27
Species * N Level	0.78	5	3.3	0.007
N Level * N Form	0.34	3	2.4	0.065
Residuals	8.54	182		

Model fit: R^2_{Adj} = 0.88, AIC = -25.09, BIC = 37.58, RMSE = 0.21

Table 2.6. ANOVA table for the best fit model examining leaf N content (%) by species and N level.

	Sum Sq	**Df**	**F-value**	**p-value**
(Intercept)	9.88	1	356.7	< 0.001
Species	0.12	1	4.3	0.04
N Level	0.53	1	19.0	< 0.001
Residuals	2.13	77		

Model Fit: R^2_{Adj} = 0.22, RMSE = 0.16

Table 2.7. Pearson's correlation between leaf N content (% N) and either leaf biomass or whole plant biomass by species, N form, and N level.

Species	**N form**	**N Level**	**Leaf Biomass**		**Whole Plant Biomass**	
			r	p-value	*r*	p-value
Acer saccharinum	Amino acids	Low	-0.89	0.008	-0.79	0.03
		High	-0.9	0.016	-0.95	0.004
	Mixture	Low	-0.55	0.2	-0.82	0.02
		High	-0.26	0.62	-0.6	0.21
	Ammonium	Low	-0.78	0.04	-0.71	0.08
		High	-0.93	0.007	-0.95	0.004
	Nitrate	Low	-0.87	0.01	-0.8	0.03
		High	-0.07	0.9	0.22	0.67
Pinus banksiana	Amino acids	Low	0.96	0.04	0.95	0.049
		High	0.9	0.29	0.89	0.3
	Mixture	Low	-0.24	0.76	-0.77	0.23
		High	-0.33	0.79	-0.31	0.8
	Ammonium	Low	0.11	0.89	-0.04	0.96
		High	-0.77	0.44	-0.91	0.27
	Nitrate	Low	0.44	0.56	0.37	0.63
		High	-0.93	0.24	-0.91	0.27

Table 2.8. ANOVA table for Leaf N (mg) by species, treatment and N level.

	Sum Sq	Df	F-value	p-value
(Intercept)	162.73	1	2960.8	< 0.0001
Species	35.59	1	647.5	< 0.0001
N Form	0.97	3	5.9	0.001
N Level	2.32	1	42.2	0.0001
Specie*N Level	0.59	1	10.8	0.002
N Form*N Level	1.13	3	6.8	0.0004
Residuals	3.85	70		

Model Fit: R^2_{Adj} = 0.94, RMSE = 0.22

Table 2.9. ANOVA table for percent colonization of roots by ECM fungi for *P. banksiana* and *P. strobus*.

	Sum Sq	**Df**	**F value**	**Pr(>F)**
(Intercept)	0.574	1	45.825	0.000
Species	0.032	1	2.519	0.118
N Level	0.129	1	10.278	0.002
N Form	0.067	3	1.782	0.161
Species * N Level	0.083	1	6.651	0.013
Species * N Form	0.026	3	0.683	0.566
N Level * N Form	0.055	3	1.465	0.234
Species * N Level * N Form	0.117	3	3.119	0.033
Residuals	0.702	56		

Model Fit: R^2_{Adj} = 0.16, RMSE =0.10

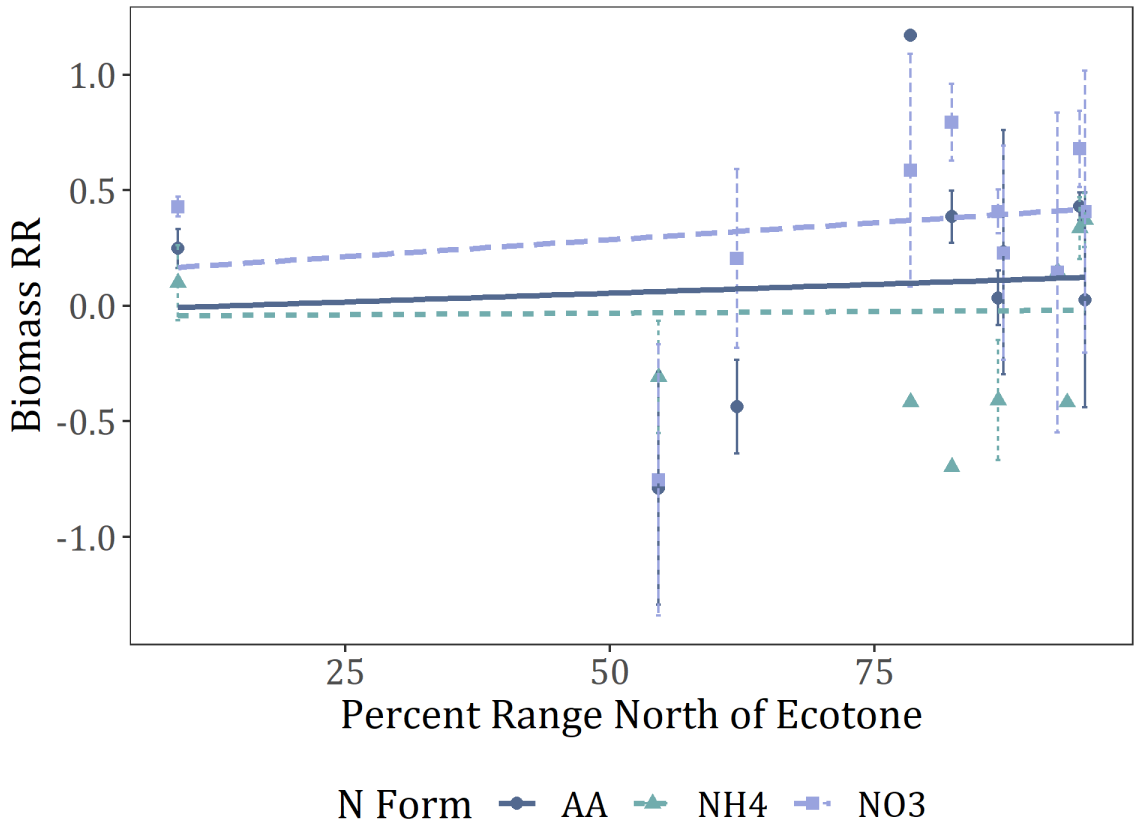

Figure 2.1. Impact of nitrogen form treatments on the growth response of species in relation to their geographic range. Results are shown for just the high N level. N form treatments are abbreviated as follows: AA = amino acids; NH4 = ammonium; NO3 = nitrate. The biomass response ratio (BRR; ln(treatment biomass/mix biomass)) for NO_3^- tended to increase with the proportion of a species' range north of the ecotone.

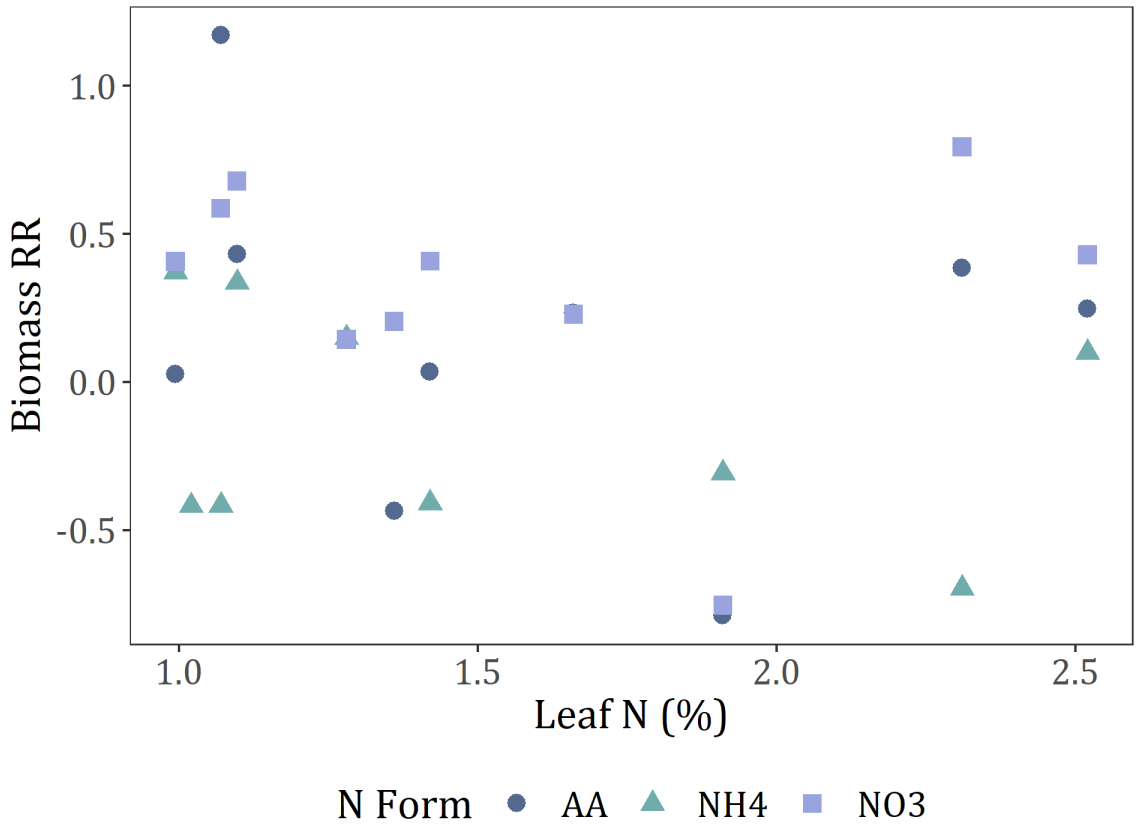

Figure 2.2. Biomass response ratio (BRR) by leaf N content for each treatment at the high N level. Points represent the mean BRR (ln(treatment biomass/mix biomass)) for each species, while leaf N % was obtained from published data for use as a covariate in this model (Wright et al. 2004). N form treatments are abbreviated as follows: AA = amino acids; NH4 = ammonium; NO3 = nitrate.

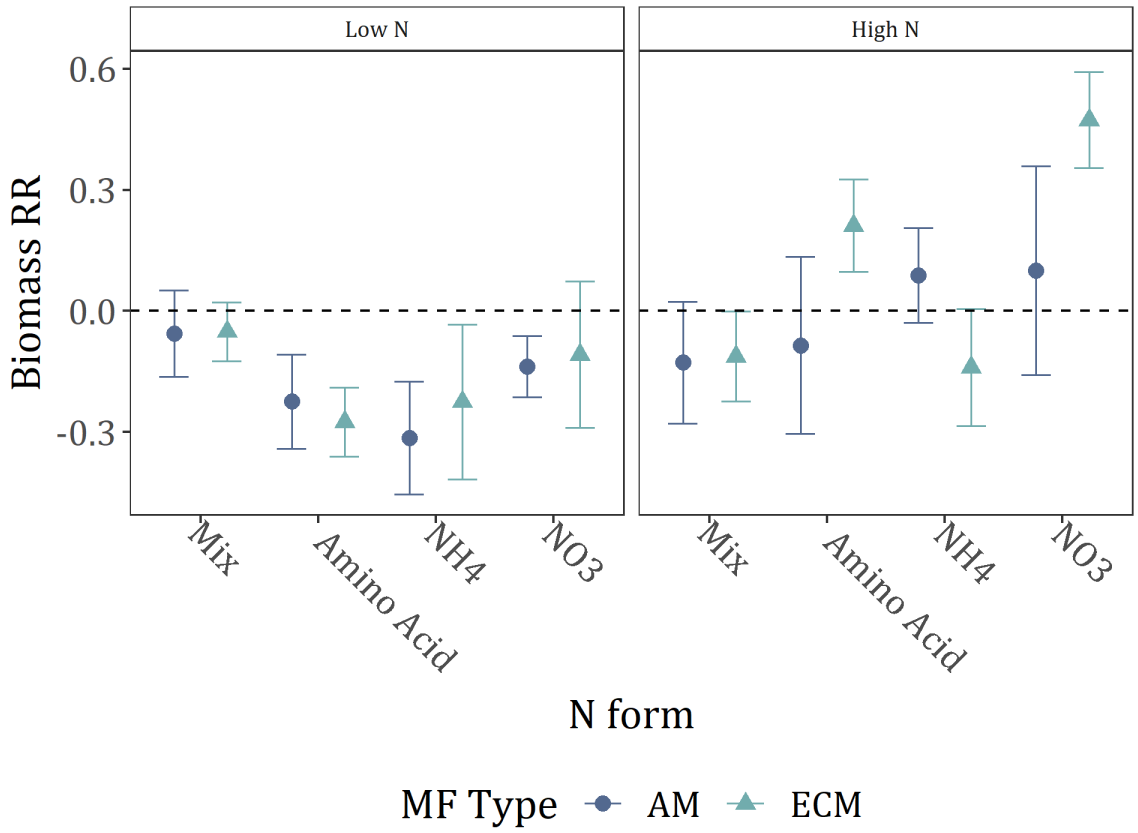

Figure 2.3. Impact of nitrogen form on the BRR (mean +/- SE) by mycorrhizal type and nitrogen level. At low N, both AM and ECM species tended to have lower BRR (ln(treatment biomass/mix biomass)) on N sources dominated by one N form than the mixture treatment, while at high N levels ECM species showed a greater biomass response on amino acids and NO_3^- compared to the mixture.

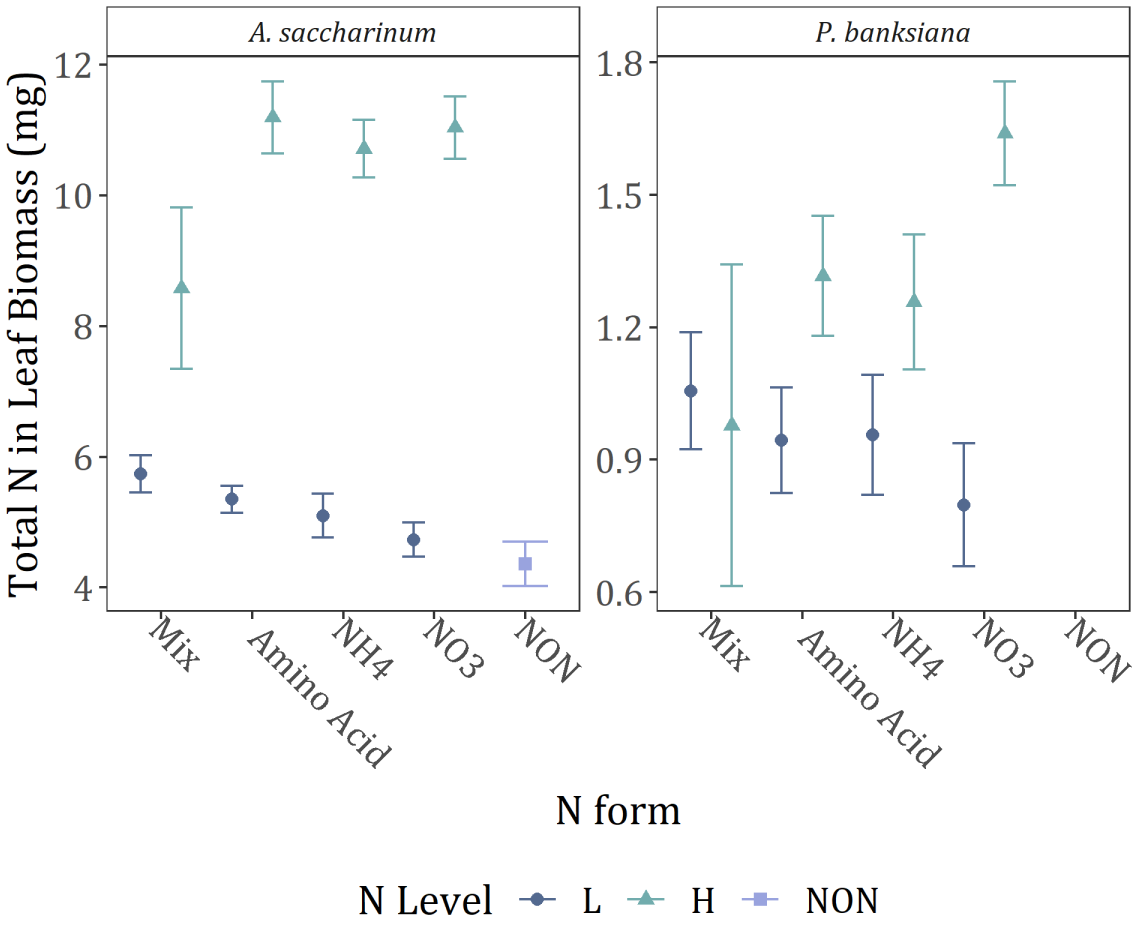

Figure 2.4. Total Leaf N in *A. saccharinum* and *P. banksiana* by N form and N level. Total leaf N is scaled to the growth period and represents the mass of N (mg) +/- SE in all leaf biomass (leaf % N * total leaf mass) for each species. Note the different scale for the two species, as *A. saccharinum* had substantially more total N than *P. banksiana* due to both higher leaf % N (Table 2.4) and overall greater biomass.

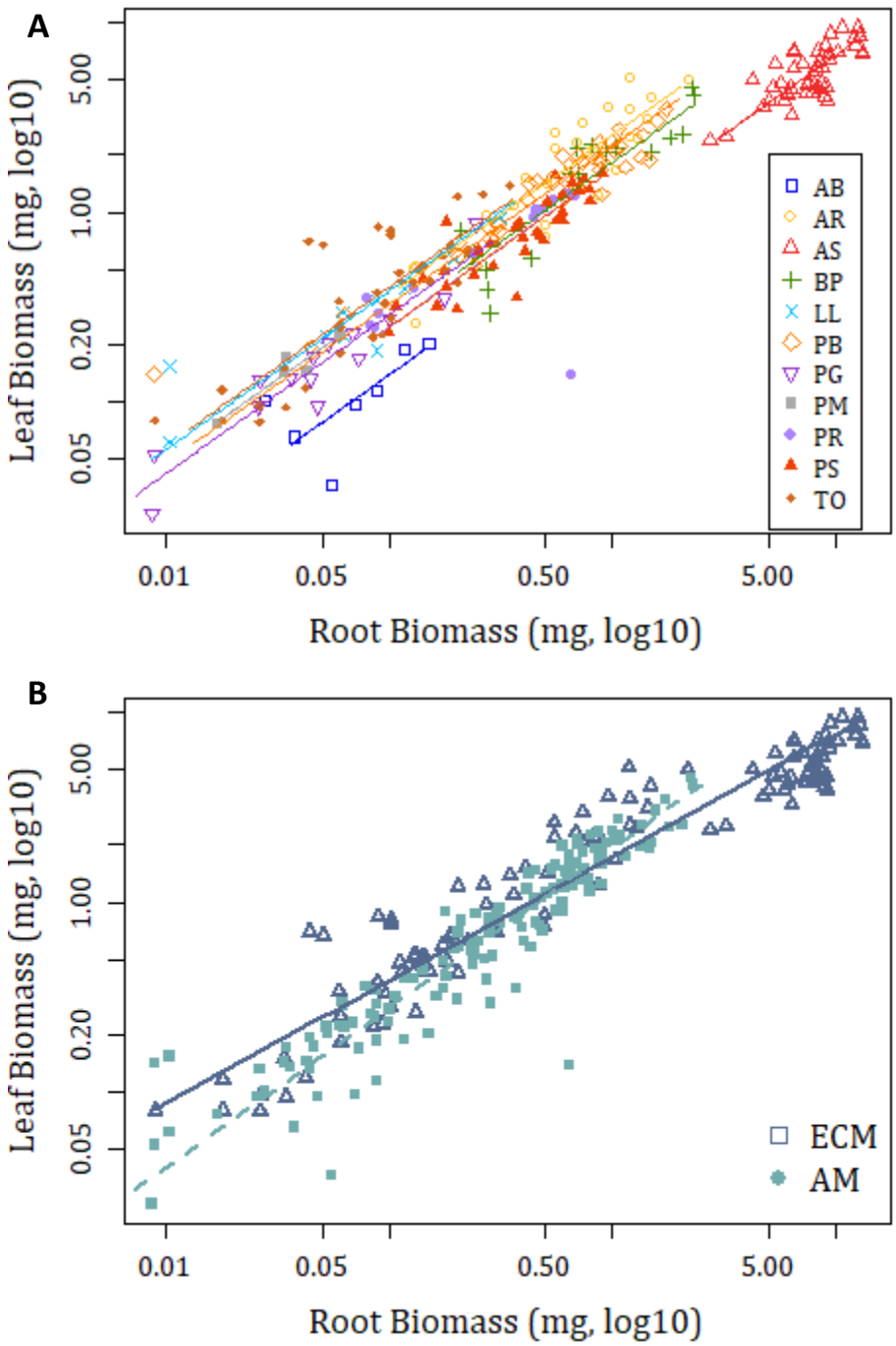

Figure 2.5. Biomass allocation by species (A) and mycorrhizal type (B) across N levels and N treatments. Species differed primarily in the overall allocation of biomass to roots versus shoots rather than the slope of that relationship. Species names are abbreviated with the first letters of the genus and species name (Table 2.1). Both ECM and AM species had greater allocation to roots versus leaves, but allocation to roots was greater in AM (slope = 0.65 (0.61 – 0.68)) species compared to ECM species (slope = 0.84 (0.79 – 0.89)) (LL ratio = 35.08, $p < 0.0001$).

CHAPTER 3

Use of different nitrogen forms by tree seedlings depends on mycorrhizal type and shows little response to experimental warming and rainfall reduction

Summary

Predicting the effects of climate change on forests will require a thorough understanding of its impacts on the supply and use of limiting resources. However, how changes in climate may alter competition for nitrogen (N), which commonly limits plant growth in northern forests, is not well understood. In particular, the factors influencing uptake of different N forms from the soil remain elusive, yet important, as climate change will likely alter the amount and types of N available to plants. At the temperate-boreal forest ecotone in North America, tree species from more southern ranges may be better poised than boreal species to take advantage of increases in inorganic N availability due to acceleration in N mineralization. Here, we examine (1) whether warming and rainfall reduction change the predominant N forms used by temperate and boreal tree seedlings at their ecotonal boundary and (2) if changes are related to a species' mycorrhizal association and/or geographic range and (3) if these changes are correlated with differences in growth. We sampled 9 tree species grown in the *Boreal Forest Warming at an Ecotone in Danger* field experiment replicated at two sites in Northern Minnesota. These species were grown under ambient or warmed (+3.2 °C) conditions crossed with ambient or reduced rainfall conditions. We sampled leaves and soil and measured their $\delta^{15}N$ signature, as well as the $\delta^{15}N$ signature of distinct soil N pools (dissolved organic N

(DON), NH_4^+ and NO_3^-) and used Bayesian stable isotope mixing models to estimate the contribution of each N form to each species' N nutrition.

For most species, NH_4^+ had a greater than 90% probability of contributing more to N uptake than either DON or NO_3^-. However, the two arbuscular mycorrhizal (AM) species had a much lower proportion of N uptake from NH_4^+ and more from DON and NO_3^- compared to the EM species. Species also varied in their responses to warming and reduced rainfall, with some showing little change in their N sources (i.e. *Acer saccharum*), while others showed reduced use of NH_4^+ (*Pinus banksiana, Quercus macrocarpa*) in reduced rainfall treatment, especially at the more northern of the two sites (HWRC). Reduced rainfall treatments had more of an effect on N sources than warming treatments in most species, especially at HWRC, with shifts towards greater NO_3^- use in ambient and dry treatments and towards DON in warm and dry treatments. These results show that species are partitioning N forms differently in the field, and that effects of climate manipulations were small relative to effects of mycorrhizal type. Thus, species may have limited capacity to alter N use to respond to changes in conditions, and therefore changes in the availability of different N forms could be a factor in impacting species' ability to respond to climate change.

Introduction

Global climate change is expected to alter the structure and function of ecosystems in the coming century (Rustad *et al.*, 2001; Bai *et al.*, 2013). While many studies have examined the consequences of projected climate change on plant growth and performance (Reich & Oleksyn, 2008; Arndal *et al.*, 2014; Reich *et al.*, 2015; Pastore *et al.*, 2020), the mechanisms underlying these responses can be complex and remain poorly characterized. For example, species can show positive responses to experimental warming due to direct stimulation of photosynthesis (Reich *et al.*, 2015), which could also be indirectly stimulated through increases in nitrogen (N) availability caused by warming (Melillo *et al.*, 2011; Bai *et al.*, 2013). Furthermore, climate change may have positive or negative impacts on the species within an ecosystem that can lead to changes in species composition (D'Amore *et al.*, 2009; Ma *et al.*, 2016).

Species' responses to climate change will likely depend in part on concurrent changes in other growth-limiting factors like soil nutrients (Rennenberg *et al.*, 2009). In many high-latitude ecosystems, N is the main limiting nutrient (Rennenberg *et al.*, 2009; Vadeboncoeur, 2010; Franklin *et al.*, 2014). Therefore, competition for N could influence species responses to climate change in these ecosystems. Warming is expected to increase rates of N cycling (Melillo *et al.*, 2011; T. Weedon *et al.*, 2012; Bai *et al.*, 2013), which would likely lead to increases in organic and especially inorganic N availability to plants (Schimel & Bennett, 2004; Dijkstra *et al.*, 2010). Furthermore, warmer temperatures can increase the rate of plant N uptake from soils (Chapin III, 1974; Chapin III & Bloom, 1976; BassiriRad, 2000). Both increases in N availability and increases in N

uptake rates could enhance the ability of some plants to access N. Drought effects on N acquisition have been studied less than temperature effects (Rennenberg *et al.*, 2009). However, existing studies show that water availability impacts N uptake through reductions in N availability, reduced uptake rates, and lower diffusion rates of N through soil to roots (Bassirirad *et al.*, 1999).

For both warming and drought, much of our knowledge regarding their effects on N use comes from experiments done in pots or microcosms or from field experiments that warmed either above or belowground. Many pot experiments have had no (or reduced) competition (Rennenberg *et al.*, 2009) and thus may have missed how the impact of climate change depends on the competitive environment. As the type of N used can be impacted by surrounding species (Miller *et al.*, 2007; Simon *et al.*, 2010), experiments that include competition along with climate manipulations are needed to better understand the impacts of climate change on species' N use. Furthermore, simultaneous warming of both above and belowground environments are needed to fully understand the impacts of warming on N use. While N uptake occurs in soil, it is influenced by whole-plant N demand and requires energy for both uptake and assimilation into plant biomass (Miller & Cramer, 2004; Britto & Kronzucker, 2006). Thus, how temperature impacts plant physiology aboveground will impact N uptake and use which would be missed by warming solely belowground. Additionally, warming and drought can have compounding effects on plant responses (Arndal *et al.*, 2014; Liu *et al.*, 2016), so experiments that solely manipulate one factor may not be able to predict how plant N use will change in the future. Thus, more experiments that include manipulations of warming

and precipitation *in situ* are needed to better understand how resource acquisition could influence community responses to climate change.

In addition to changes in total N availability, warming and drought can influence the forms of N that are available and whether they are taken up by plants. Plants can acquire N in several forms (e.g. dissolved organic N (DON), NH_4^+, and NO_3^-), each with different transporters for uptake and costs of assimilation (Miller & Cramer, 2004; Franklin *et al.*, 2017). Warming and drought can impact the uptake and assimilation of each form differently as N forms differ in the temperature sensitivity of their uptake rates. NO_3^- uptake rates tend to peak at higher temperatures than NH_4^+ uptake rates (Reay *et al.*, 1999; Boczulak *et al.*, 2014); consequently, warming could increase the uptake of NO_3^- compared to dissolved organic N (DON) or NH_4^+. Drought may favor the uptake of different N forms than warming. For example, drought can decrease NO_3^- availability (Smith *et al.*, 2003; Miller *et al.*, 2005), which may promote the use of other N forms. However, the responses of the microbial community to drought are complex and other studies have shown increases in the pool sizes of inorganic N (He & Dijkstra, 2014). Growth responses could also vary with different N forms under drought. New evidence is emerging that organic N may improve tree seedling acclimation to drought (Sigala *et al.*, 2020), making DON a more beneficial N source in drought conditions. Drought may also impact N acquisition through reductions in fine root biomass (Valdés *et al.*, 2006), reducing the total area for N absorption, as well as reducing rates of diffusion of N to the root surface (Clarke & Barley, 1968). Reducing the rate of N supply to the root surface could make differences in uptake rates between forms less important, as N uptake would be more limited by the availability of N than the capacity for uptake (Chapin III, 1983).

Though the previous studies have shown that both warming and drought can impact the acquisition of different N forms, whether these effects are consistent when warming and drought are applied together and when plants are competing with each other is not well understood.

Determining whether climate change will impact the forms of N used by plants is important because species can differ in their growth on different N forms. Though some species have shown equivalent growth on organic N, NH_4^+, and NO_3^- (Öhlund & Näsholm, 2001), many others show an increase in their growth on one form over another (Persson *et al.*, 2006; Boczulak *et al.*, 2014; Franklin *et al.*, 2017; King *et al.*- Chapter 2). The N form that is most beneficial to a particular species can also change depending on the environmental conditions (Sigala *et al.*, 2020). Additionally, the type of N supplied as well as total N availability can impact the outcome of competition for N, varying the predominant N form used by a species as well as its growth in competition compared to growing alone (Simon *et al.*, 2011; Li *et al.*, 2015). These studies suggest that species will respond differently to potential changes in the availability of different N forms caused by warming and drought.

How species will respond to climate change in terms of N uptake, and potentially growth, could depend on their traits associated with nutrient uptake. For example, species from warmer climates tend to have N uptake rates that peak at warmer temperatures (Boczulak *et al.*, 2014), which may make them more competitive for all N forms with warming than species from cooler climates (Chapin III, 1974; Chapin III & Bloom, 1976; BassiriRad, 2000; Boczulak *et al.*, 2014). Less competitive species may then be relegated

to using other N forms that could reduce either their total N acquisition or increase the costs of N uptake, both of which could negatively impact their growth (McKane *et al.*, 2002). Additionally, mycorrhizal type can impact the forms of N that a plant acquires. Species that associate with ectomycorrhizal fungi (EM) are often better at acquiring organic N or NH_4^+ than NO_3^- in comparison to species that associate with arbuscular mycorrhizal fungi (AM) (Rains & Bledsoe, 2007). Warming could therefore favor the growth of AM species if they are better able to take advantage of inorganic N resources. As stated previously, the reductions in soil moisture caused by warming and/or reduced rainfall may reduce the importance of differences in uptake rates between species and N forms. Instead, drought may favor species with thinner roots and greater root length density that enable them to maintain access to soil resources at lower soil moisture (Comas *et al.*, 2013).

Increasing our understanding of how climate change impacts the partitioning of N resources in the field can reduce uncertainty around plant and ecosystem responses to climate change. Therefore, the major objective of this study was to identify whether species differ in their use of N forms *in situ*, and if warming and drought alter these patterns. We also examined if there were patterns in N source use or responses to climate by mycorrhizal type or species range limit. Finally, we evaluate whether changes in the N source used by plants are associated with changes in growth, and if the effect size or direction varies by mycorrhizal type and species range limits.

We hypothesized that species would differ in the forms of N used in ambient conditions, with AM species using more inorganic N forms than EM species (Table 3.1),

and species from warmer climates using more NH_4^+ than DON in comparison to species from cooler climates. We further hypothesized that warming would enhance the uptake of NO_3^- compared to the other N forms, especially in AM species and species with warmer ranges, but that drought would reduce NO_3^- uptake across all species. We also hypothesized that changes in N sources with warming and drought would be associated with changes in growth and depend on the traits of species. Specifically, changes in the type of N used would be associated with increases in growth for AM and/or temperate species with warming since they would favor increased N uptake of N forms they are better able to use, while responses would be negative for EM and/or boreal species since they may be less competitive for their preferred form. In contrast, with drought changes in N form may be more neutral in terms of plant growth across species and may become negative for all species with warming and drought in combination.

To test these hypotheses, we examined the impacts of warming and rainfall reduction on tree seedlings in the *Boreal Forest Warming at an Ecotone in Danger* (B4WarmED) project at the temperate-boreal forest ecotone in Northern Minnesota. This is an ideal location to examine how climate change may impact N acquisition, as it includes species that are likely to differ in their temperature optima for N uptake (i.e. temperate vs. boreal species) as well as species that may prefer different forms of N (i.e. AM vs. EM species). We estimated the contributions of each soil N pool to plant N use with stable isotope mixing models. Soil N pools and leaves were sampled to obtain the input to these models, and at the end of the growing season we measured the diameter and height of each tree to estimate its total biomass.

Methods

Study site and experimental design

All sampling was conducted within the B4WarmED project in northern Minnesota, USA (Rich *et al.*, 2015). This project manipulates temperature and precipitation to study climate change effects on tree regeneration and ecosystem processes at the temperate-boreal forest ecotone. The project is located at two sites, one at the Hubachek Wilderness Research Center (HWRC), Ely, MN (47° 56’ 46” N, 91° 45’ 29” W) and the other at the Cloquet Forestry Center (CFC), Cloquet, MN (46° 40’ 46” N, 92° 31’ 12” W), both of which are operated by the University of Minnesota. The CFC site is slightly warmer and wetter (4.5 °C MAT, 807 mm MAP) than HWRC (3.0 °C, 722 mm MAP), while at both sites experimental plots are located on coarse-textured upland soils (Rich *et al.*, 2015). *B4WarmED* is set up in a block design, with a complete factorial of warming (3 levels: ambient, +1.7°C, + 3.4°C) crossed with precipitation reduction (2 levels: ambient, 40% removal) and site (2 levels: CFC, HWRC) for a total of 12 treatment combinations. Each site has three blocks with each treatment combination (warming*precipitation) applied over one 3-m diameter circular plot. The warming treatment uses infrared heat lamps and soil heating cables to warm air, plant and soil temperatures by +1.7°C and +3.4°C above ambient. Temperature is continuously monitored to maintain this differential throughout the entire growing season, and warming is applied only during the growing season, defined as air temperatures climbing above or below 1 °C for more than 5 consecutive days. Rainout shelters were put in place

for different rain events to remove ~40% of the precipitation throughout the growing season relative to ambient plots. For this study we excluded the medium heat treatment (+1.7°C) from our sampling, but sampled across the remaining heat and precipitation treatments at both sites (8 treatments total). In May – September 2015, temperatures were maintained at +3.2 °C and +3.4 °C aboveground at CFC and HWRC, respectively, and at +3.3 °C belowground at both sites. For the same time period precipitation in ambient plots was near the 75th percentile and 50th percentile of rainfall for the previous 100 years at HWRC and CFC, respectively. Thus, the 40% reduced rainfall resulted in a moderately dry environment at CFC relative to the 100-year data and a very dry environment at HWRC. Plots were located in an area formerly dominated by aspen-birch (*Populus tremuloides*-*Betula papyrifera*) forest that was cleared in winter 2006/2007 (Cloquet) and early spring 2008 (HWRC).

Within each plot, 121 seedlings from ten tree species were planted in a gridded design. Seedlings sampled in this study were planted in 2012 or 2013. Each species planted in the experiment is common at the temperate-boreal forest ecotone in northern MN and has different geographic range limits and plant traits (Table 3.2). Only nine species were sampled in this experiment due to high mortality of one of one of the species (*Populus tremuloides*) across all treatments.

Field sampling

Leaf and soil samples for isotopic analysis were collected from each plot near the end of the growing season in 2015 (late summer/early fall). For each tree species we

sampled four sun-leaves from the current year's growth, one in each cardinal direction. Leaves were composited into one sample for each plot and kept cool at 4 °C until they could be dried. Soil samples were collected around the same time as leaf samples for bulk soil $\delta^{15}N$, soil %N, and for $\delta^{15}N$ of the extractable soil N pools. In each plot, soil was sampled with a 5 cm diameter soil corer to a depth of 10 cm at two locations and the composited in the field. Samples were kept on ice and then at 4 °C prior to analysis.

Growth data for individuals was collected during the annual census of the experiment. Tree diameter and height were measured at the end of the growing season and used to calculate total stem biomass (Reich et al. 2015). Growth measurements on individual trees from 2015 were mapped to leaf $\delta^{15}N$ values to determine whether changes in growth could be correlated to changes in N source used by each individual. We used growth measurements from the 2015 growing season to better track the N sources identified in leaf samples from the same year.

We also determined the average percent colonization of root tips by ectomycorrhizal fungi for two tree species. EM fungi tend to transfer N that is depleted in ^{15}N relative to the source (Hobbie & Hobbie, 2006), and since the percent colonization of root tips can be indicative of the amount of N transferred through the EM fungi (Hobbie & Colpaert, 2003) we used the % colonization to help parameterize the mixing model used to partition N sources. Whole root systems of *P. glauca* and *Q. rubra* were excavated in fall 2016 and brought back to the lab for processing. Roots were washed with deionized water and fine roots were trimmed off the main root system. We initially scored the entire root system of three individuals per species to determine the minimum

number of root tips that had to be counted to reasonably estimate the percent colonization of the whole root system. We then scored this fraction of the remaining root system by clipping roots into smaller sections and separating them into 9 subsamples which were randomly selected for scoring. Typically 4-5 subsamples were scored before reaching the appropriate number of root tips to estimate the percent colonization of the whole root system. Percent colonization was calculated as the number of ECM root tips/total root tips x 100%.

Laboratory analyses

Leaf samples were dried at 60 °C for 48 hrs and then ground to a fine powder, packed into tin capsules (Costech 5 mm x 9mm) and analyzed for $\delta^{15}N$ and leaf N % at the UC Davis Stable Isotope Facility using a Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) interfaced to an Isoprime VisION IRMS (Elementar UK Ltd, Cheadle, UK). Fresh soils were sieved (2 mm) and separated into subsamples for different analyses. Soil moisture was determined on one 10 g subsample by drying at 105 °C for 48 hrs, while bulk soil for $\delta^{15}N$ analysis was dried at 60 °C for 48 hrs. Bulk soil was ground to a fine powder and packed into tin capsules for $\delta\ ^{15}N$ and %N analysis at the Cornell Stable Isotope Laboratory (COIL), Ithaca, NY. Remaining soil that was not used for the extractions below was air-dried and archived.

We obtained soil extracts for isotopic of analysis of each soil N pool by shaking 20 g of fresh, sieved soil for 1 hr with 100 mL of 0.5 M K_2SO_4. These extracts were then filtered through Whatman No. 1 filter circles. Filters were pre-leached to remove

potential N contamination by rinsing 3X with ultrapure DI water, 3X with 0.5 M K_2SO_4, and 3X with ultrapure DI water, drawing off each time with vacuum filtration. Soil extracts were frozen at -20 °C until analysis. We determined the concentration of each N pool with colorimetric analyses for each form, using the salicylate method for NH_4^+ (Verdouw *et al.*, 1978), $VaCl_3$ method for NO_3^+ (Doane & Horwáth, 2003), and persulfate digestion to convert DON to NO_3^- followed by the $VaCl_3$ method to determine total dissolved N (Stark & Hart, 1996). DON was then calculated as the difference between TDN and the sum of the NH_4^+ and NO_3^- pools.

The isotopic signature of each pool was determined using a diffusion protocol on soil extracts (Stark & Hart, 1996). Diffusions were set up with 30 mL of soil extract in 120 mL specimen cups (Simport Eco-Friendly SpecTainer), with NH_4^+ and NO_3^- $\delta^{15}N$ determined from one sequential diffusion and DON $\delta^{15}N$ determined in a separate diffusion after the persulfate digestion. All diffusions were spiked with an additional 20 µg of N (NH_4NO_3) due to low concentrations in the soil extracts. Acid traps for the diffusion were constructed from ¾” (1.91 cm) Teflon tape that enclosed two 1cm diameter glass fiber filter disks (Whatman GF/D) that had been heated to 400 °C to remove N contamination. 10 µL of 2.5 M $KHSO_4$ was pipetted onto each disk prior to sealing the disks between layers of the Teflon tape and placing them in the extracts. Ammonium was collected by adding 0.2 g MgO to specimen cups along with an acid trap and diffused for 6 days with gentle shaking. Following diffusion of NH_4^+ the acid traps were removed and dried in a dessicator with an open container of concentrated sulfuric acid to prevent atmospheric deposition of N onto the filter disks. For NO_3^- diffusions, another 0.2 g MgO were added to the specimen cups along with 0.3 g Devarda’s Alloy to

reduce NO_3^- to NH_4^+ and diffused for 6 days. The acid traps were removed and dried with the same procedure. Diffused and undiffused standards were run alongside the soil extracts to check for complete diffusion of N in the samples. Diffusions of DON followed the same protocol as the NO_3^- samples but were run on soil extracts after digestion with a persulfate reagent to convert DON + NH_4^+ to NO_3^-. All acidified disks were packed into tin capsules after drying and analyzed for $\delta^{15}N$ and %N at COIL.

Calculations and statistical analysis

Isotope values are reported using the delta notation describing the isotopic composition of the sample relative to a standard (Dawson *et al.*, 2002). To determine the $\delta^{15}N$ of each soil N pool we needed to correct for the addition of the spike and N contamination from the salt solution, as well as contamination from persulfate for DON, using blanks of 0.5 M K_2SO_4 from the initial soil extractions that were diffused alongside the samples. We used the following equation after Stark & Hart (1996) to correct our measured $\delta^{15}N$ value:

$$\delta^{15}N_{corr} = \frac{\delta^{15}N_t M_t - \delta^{15}N_b M_b - \delta^{15}N_{sp} M_{sp}}{M_t - M_b - M_{sp}}$$

where $\delta^{15}N_t$ and M_t are the isotopic signature and mass of N for the sample, spike, and blank combined, respectively; $\delta^{15}N_b$ and M_b are the isotopic signature and N mass in the blank; and $\delta^{15}N_{sp}$ and M_{sp} are the isotopic signature and N mass of the spike. For DON, the mass and enrichment of the persulfate solution were included in the $\delta^{15}N_b$ and M_b. We

also had to calculate an adjusted DON $\delta^{15}N$ signature based on the relative concentrations of NH_4^+ and NO_3^- in each sample based on the following equation:

$$\delta^{15}N_{DON} = \frac{\delta^{15}N_{corr}M_t - \delta^{15}N_{NH4}M_{NH4} - \delta^{15}N_{NO3}M_{NO3}}{M_t - M_{NH4} - M_{NO3}}$$

We calculated the proportion of each N form contributing to a species' N use using Bayesian stable isotope mixing models (Parnell *et al.*, 2013). For this analysis, we used the *simmr* package (v. 0.4.2) in R. We calculated the mean $\delta^{15}N$ value for N form in each treatment at each site to use as the input for the $\delta^{15}N$ of the source. The mean concentration of each N form in each treatment was also used to account for potential concentration dependence of N uptake. We used different enrichment factors for arbuscular mycorrhizal and ectomycorrhizal species, as they differ in the fractionation of N that they pass along to their host (-1 ‰ for AM (Handley *et al.*, 1993), -8-10 ‰ for EM (Hobbie & Colpaert, 2003)). After running the mixing model, we compared the probability that one N form was the predominant N source for each species. Since both DON and NO_3^- tended to have similar isotopic signatures, we primarily compared the use of NH_4^+ across species since this pool was highly enriched relative to the other two forms. We used the mean proportion of N coming from NH_4^+ in additional statistical analyses and to calculate the change in NH_4^+ use in treatments relative to ambient conditions for comparing whether changes in growth could be correlated to changes in NH_4^+ use.

All statistical analyses were conducted in R version 4.0.0 (R Core Team, 2020). We first checked for outliers in the data using the *outliers* package (v. 0.14) and found variables that contained values greater than 99% of others using the chi-square test. We

then examined Cleveland Dot Plots for those variables to see if there were extreme high or low values that were likely to influence our analyses (Zuur *et al.*, 2007). A small number of $\delta^{15}N$ values for NH_4^+ at very low NH_4^+ concentrations were highly depleted relative to other $\delta^{15}N$ values for this pool so we excluded them from the calculation of the mean $\delta^{15}N$ value for their respective treatments and comparisons of $\delta^{15}N$ by treatment and site.

We used a combination of linear models and linear mixed effects models to compare the impact of warming and precipitation reduction on leaf and soil measurements of $\delta^{15}N$ and % N. We also tested whether warming and precipitation altered the proportion of N coming from NH_4^+ and if the response varied by species traits (mycorrhizal type, range limit). We performed backwards model selection using the *stepAIC* function (*MASS* package v. 7.3-51.5) on models fit with *lm* or *lme* (*nlme* package v. 3.1-147) functions to find the best model for each dependent variable with Heat, Water, Site, and their interactions as the predictors. When necessary, we included random effects for Block nested within Site and Species nested within Block. After finding the best model, we summarized the effects using the *Anova* function (*car* package v. 3.0-7) to run a type III SS analysis of variance to test whether N form or N level influenced growth by species. We followed up with pairwise comparisons for significant predictors using the *emmeans* function (*emmeans* package v. 1.4.6) with the Tukey adjustment for multiple tests. Models were checked to ensure that they did not violate key assumptions using the *check_model* function (*performance* package v. 0.4.7).

Results

Leaf $\delta^{15}N$ values vary by species and treatment, soil $\delta^{15}N$ values vary by site and form

Leaf $\delta^{15}N$ values differed more between species than between either warming or rainfall reduction treatments (Fig. S3.1). For example, differences between treatments were estimated to be at most 0.5 – 1 ‰, while differences between species could be greater than 4 ‰. However, incorporating the effects of warming and rainfall reduction did improve the fit of the mixed-effects model from one with just species and site, though the improvement was small ($\Delta AIC = 7.8$, $R^2_{cond} = 0.56$ vs. 0.53, Table S3.2). Both warming and rainfall reduction tended to enrich leaf $\delta^{15}N$ relative to ambient plots, though for both treatments the effect was larger at HWRC compared to CFC (Table S3.3, S3.4). In contrast, species differences in leaf $\delta^{15}N$ did not vary by site, with *A. balsamea* tending to have the most enriched $\delta^{15}N$ values across sites, especially compared to *A. rubrum* and *A. saccharum* ($t = 7.66$, $df = 38.5$, $p < 0.0001$; $t = 6.53$, $df = 38.6$, $p < 0.0001$; respectively).

There were small differences in bulk soil N and $\delta^{15}N$ by site and treatment, though treatment effects were not usually significant (Table S3.5). Bulk soil %N tended to be higher at CFC but only marginally ($t = 1.14$, $p = 0.27$). The $\delta^{15}N$ of the bulk soil was on average slightly enriched at HWRC compared to CFC ($t = -3.53$, $p = 0.002$) but least so in the warm-reduced rainfall treatment at CFC. Similarly, the concentrations of each N form in soil solution did not vary by treatment but did vary by site and N form (Table S3.6, S3.7). Concentrations of DON, NH_4^+ and NO_3^- were similar at CFC ($p > 0.37$ for all), but not HWRC (Fig. S3.2). At HWRC, DON concentrations were ~7.5X

and 6X higher than NH_4^+ and NO_3^-, respectively ($p < 0.0001$ for both). This was due to lower concentrations of NH_4^+ and NO_3^- at HWRC compared to CFC ($p = 0.0008$ and $p = 0.003$, respectively), since DON concentrations were similar at the two sites ($p = 0.55$).

The $\delta^{15}N$ of soil N pools varied primarily by site and N form but not by heat or water treatments (Table S3.8). In all treatments, NH_4^+ was enriched relative to both DON and NO_3^- ($p < 0.0001$ for both pools and sites), and DON and NO_3^- had similar $\delta^{15}N$ signatures (CFC: $t = -0.64$, $p = 0.80$; HWRC: $t = 2.02$, $p = 0.11$, Fig S3.3). At HWRC, NO_3^- tended to be more depleted in ^{15}N than at CFC ($t = 1.63$, $p = 0.11$), and so the difference between the NH_4^+ and NO_3^- pools was larger at HWRC (24.81‰ vs. 15.29‰). Given the similarity in the $\delta^{15}N$ of DON and NO_3^-, which limits the ability of the mixing model to partition N between the two potential sources, we focus on comparing the effects of warming and drought on the proportion of N coming from NH_4^+ which had a distinct $\delta^{15}N$ signature.

Effects of warming and precipitation reduction on N source partitioning

NH_4^+ was the predominant N form used by all but two species at both sites, while warming and precipitation treatments tended to have small but consistent reductions in species' reliance on NH_4^+. The two maples had roughly similar proportions of N coming from DON, NH_4^+, or NO_3^- (Fig. 3.1, 3.2), while the median estimates for the other seven species were usually > 50% of N from NH_4^+, and up to around 75% for a few species (e.g. *A. balsamea*, *P. banksiana*). The patterns were consistent across sites, but overall NH_4^+contributions were lower at HWRC than at CFC for most species. For six (five) of

the nine species at CFC (HWRC), the probability that NH_4^+contributed more to their N source than the other two forms was > 0.8 in ambient plots (Table 3.3). At both sites, warming had less of an effect than rainfall reduction on the proportion of NH_4^+ used. For most species, the probability that NH_4^+ was the predominant source remained high in both warmed and ambient plots. Rainfall reduction tended to decrease the contribution of NH_4^+ to a species N source except for *A. rubrum* and *A. saccharum*, which had slightly higher NH_4^+contributions in ambient-dry conditions at CFC and similar contributions across treatments at HWRC. The effect of reduced rainfall was also stronger at HWRC than at CFC, with larger reductions in the estimate of the NH_4^+contribution to species N uptake (Fig. 3.2). Coupled with the reductions in NH_4^+ use, species increased their use of either DON or NO_3^- with warming and reduced rainfall, and the estimates for DON use increased more at HWRC than at CFC.

The effect of warming and rainfall reduction on N use depends on mycorrhizal type

The effect of warming and rainfall reduction on the proportion of N coming from NH_4^+ depended on mycorrhizal type but not species' range (Fig. 3.3). While species' range and mycorrhizal type both helped explain variation in the use of NH_4^+ (Table 3.4), only mycorrhizal association interacted with the treatments (Table 3.5). The effect of rainfall reduction depended on both MF type and site: EM species used less NH_4^+ in dry plots compared to ambient at both CFC ($p = 0.017$) and HWRC ($p < 0.0001$), and the effect of rainfall reduction was stronger at HWRC (~5% change at CFC vs. ~12% at HWRC). AM species were relatively insensitive to heat and reduced rainfall treatments,

though they tended to use more NH_4^+ in dry compared to ambient conditions at HWRC. While range helped explain variation in species' use of NH_4^+, its effect did not vary with warming or rainfall reduction. Similarly, the heat treatment impacted species' N use, but its effect did not depend on mycorrhizal type or range. Though the model explained a large amount of variation in the data ($R^2_{adj} = 0.77$), it should be noted that the dependent variable was estimated from the mixing model with large confidence intervals.

Changes in N use are weakly related to changes in leaf N and growth

Leaf N content varied more across species than across treatments. Across treatments, the two oaks (*Q. macrocarpa, Q. rubra*) and *B. papyrifera* had the highest leaf %N, while *P. glauca* and the two maples had the lowest leaf %N (Figure 3.4). There were small effects of heat on leaf %N, with warming tending to increase leaf %N from ambient ($t = 1.86$, $p = 0.06$). Reducing precipitation did not consistently affect leaf %N, and there was no interaction between warming and precipitation reduction (Table S3.9). Differences in leaf %N between ambient and treatment plots were sometimes associated with changes in N source. At HWRC, treatments tended to increase leaf N content relative to ambient conditions, and for EM species, especially temperate forest species, this was associated with decreases with NH_4^+ use in dry conditions (Fig S3.4). AM species had more variable leaf %N at HWRC, but warming tended to decrease leaf %N and was associated with a slight increase in N from NH_4^+. Changes in leaf %N at CFC were smaller and did not clearly map onto changes in NH_4^+ use in any treatment.

Growth of AM species responded more positively to treatments than EM species, and growth was inconsistently associated with increases in the use of one N source for both groups. At CFC, AM species in warm-dry treatments had greater stem mass than ambient plots and lower NH_4^+ use (Figure S3.5), while warm-ambient treatments had positive growth with no change (CFC) or an increase (HWRC) in NH_4^+ use. EM boreal species had consistently lower growth in all treatment plots at CFC compared to ambient, which coincided with consistent reductions in NH_4^+ use. However, at HWRC reductions in NH_4^+ use were associated with more growth in ambient-dry plots and less growth in warm-dry plots. EM temperate species had more variation in their growth at CFC, but tended to have increased growth and decreased NH_4^+ use in all treatments. In contrast, at HWRC EM temperate species had lower growth in all treatments and lower NH_4^+ use, with the greatest declines in both growth and NH_4^+ use in the reduced rainfall treatments.

Discussion

Species differ in their use of N forms

Our experiment tested the effects of warming and reduced rainfall on tree seedling N use and examined whether these impacts varied by mycorrhizal type and species' range. We also looked for differences between species in their use of different N sources to see if species differ in how they partition N under current climate conditions. We found that tree seedlings differed in their use of N forms, with EM species obtaining most of their N from NH_4^+. In contrast, AM species had a much lower reliance on NH_4^+ and used N forms in more equal proportions than EM species. While this matched our

hypothesis for EM temperate species, which we had expected to rely on NH_4^+, EM boreal species had higher estimates for the amount of N derived from NH_4^+ compared to temperate species, rather than using more DON than NH_4^+ as we had expected. However, most N uptake studies have shown that species similar to those in our study readily take up NH_4^+(Kronzucker *et al.*, 1997, 2003; Öhlund & Näsholm, 2004; Britto & Kronzucker, 2013), and that uptake of NH_4^+ can exceed that of organic N as well (Scott & Rothstein, 2011). The lower than expected contribution of DON could be related to the availability of each form; we had expected DON concentrations to be greater than both inorganic N forms, but NH_4^+ and DON had similar soil concentrations at CFC. This would also concur with our results from HWRC, where DON concentrations were higher than NH_4^+ concentrations in soil and NH_4^+ contributed less to the N used by these species. For example, two of the EM boreal species (*B. papyrifera* and *P. glauca*) had lower estimates for the amount of N coming from NH_4^+ at HWRC, closer to 50% rather than 75%. Additionally, while DON may have been in slightly higher abundance than other N forms in some sites and treatments, it is possible that it was available in forms that plants were less able to take up (Näsholm *et al.*, 2009), or that they were outcompeted for DON by soil microbes (Harrison *et al.*, 2007).

The roughly equitable use of soil N forms by AM species suggests that, in total, they use a greater proportion of N from NH_4^+ and NO_3^- compared to DON, in accordance with our hypothesis. Additionally, the difference between EM and AM species in the use of N forms suggests that species are partitioning N in our system, rather than all species using each N form as it is available. It is perhaps surprising that AM species did not take up more NH_4^+, as they should have adequate capacity for its uptake (Socci & Templer,

2011) and both AM species in this study have more temperate ranges. However, this could be due to the competitive environment (Miller *et al.*, 2007), and studies have shown that in competition, *Acer* spp. may not compete as strongly for NH_4^+ compared to EM species (Nave *et al.*, 2013). Since most of the species in the plots were EM, they may have outcompeted the AM species for NH_4^+ and increased their reliance on NO_3^-. The AM species in our study may also have had similar uptake rates for NO_3^-, DON and NH_4^+ that would help explain their more equitable use of the three forms; however, other studies of *A. saccharum* have shown that it tends to have lower or negligible uptake of DON (King *et al.* unpublished – Ch. 1) and NO_3^- (Rothstein *et al.*, 1996; Socci & Templer, 2011) compared to NH_4^+. Other maple and AM species have shown significant capacity to use DON (Whiteside *et al.*, 2012; King *et al.*, unpublished - Ch. 1), so the uptake of DON in these species is not without precedent.

Combined, our results imply that there is some partitioning of N resources by species of different mycorrhizal associations. Though the EM species seem to be using the same N resources, we could also be missing different ways they are partitioning N. For example, species can also partition N temporally by taking up N in different seasons (McKane *et al.*, 2002; Pornon *et al.*, 2007; Socci & Templer, 2011) as well spatially by differing in the depth of N uptake (McKane *et al.*, 2002; Houle *et al.*, 2014). Since our sampling of leaf $\delta^{15}N$ is an integrated measure of the forms of N used by an individual over the course of the growing season, we cannot determine whether there is any temporal separation in N use by species in our experiment.

Rainfall reduction has a greater effect than warming on use of NH_4^+

Rainfall reduction impacted the proportion of NH_4^+ used by species more so than warming treatments, but only for EM species. Additionally, the variation between treatments in the use of N forms was small compared to the differences between AM and EM species. The reduction of NH_4^+ use with rainfall reduction supports our hypothesis that species would become more similar in their N use, since the species with reduced NH_4^+ use had high NH_4^+ use initially. Drier conditions would potentially limit N supply to plant roots (Rennenberg *et al.*, 2009), which would make N uptake of different forms more similar since uptake rates for all N forms may outpace the supply of N to roots. There were also small increases in the estimates of DON use, especially in the warm-reduced rainfall treatment, which concurred with our hypothesis. However, we did not see a decline in NO_3^- use with rainfall reduction, and at HWRC the estimated contribution of NO_3^- actually increased with rainfall reduction for EM species. This could in part be due to the overlap of DON and NO_3^- in isospace, which makes it difficult to accurately partition whether a species was taking up DON versus NO_3^-. Additionally, our hypotheses relied on the assumption that rainfall reduction would cause drought-like conditions that would reduce the availability of NO_3^- (Smith *et al.*, 2003; Miller *et al.*, 2005). However, it is possible that even with 40% rainfall reduction the plots may not have experienced drought conditions. Rainfall at CFC during the growing season was above average for the 100-year period prior (~75th percentile), so reduced rainfall would not likely have produced intense drought conditions. Instead, the slightly drier conditions may have facilitated NO_3^- uptake as it diffuses more readily through soil compared to NH_4^+ and DON (Miller & Cramer, 2004). However, at HWRC, growing season rainfall

was closer to the 100-year mean so reduced rainfall treatments would have been drier than at CFC. This also aligns with the reduced rainfall treatments showing a larger impact on NH_4^+ use and increase in NO_3^- use at HWRC compared to CFC. We also did not notice any declines in NO_3^- concentrations with the rainfall reduction, so it is possible that NO_3^- availability was not lower in these plots. NO_3^- availability could also have increased in the ambient temperature-reduced rainfall treatments, as the difference in enrichment between the NH_4^+ and NO_3^- pools was highest in those plots, which is indicative of higher rates of nitrification (Brearley, 2013). This would concur with the slight increase in NO_3^- contributions in the reduced rainfall plots.

The minor impacts of heat alone on NH_4^+ use were not aligned with our initial expectations. In warm-ambient rainfall plots the probability that NH_4^+ was the dominant N form used remained high for most species. Additionally, at HWRC, though warming did not substantially alter the proportion of NH_4^+ taken up, the probability that more NH_4^+ was used than the other forms increased. The insensitivity of species to the heat treatments was more expected for the boreal species, as they may have already reached their temperature optima for N uptake rates across forms. A study of three conifers varying in their geographic range found that N uptake rates of the cooler species did not increase with temperatures above 10 °C (Boczulak *et al.*, 2014), which is below the average growing season soil temperatures in ambient plots at CFC and HWRC (10.5 – 13 °C for 2009-2011 (Rich *et al.*, 2015)). Similarly, NO_3^- uptake only reached < 16% its maximum capacity in two similar tree species (Gessler *et al.*, 1998). Therefore, it is possible that temperatures were not high enough to see an increase in NO_3^- uptake, since NO_3^- uptake in previous studies did not peak and/or surpass NH_4^+ uptake rates until soil

temperatures reach ~20-25 °C (Gessler *et al.*, 1998; Vaast *et al.*, 1998; Boczulak *et al.*, 2014). So, even if NO_3^- uptake rates were increasing, NH_4^+ uptake may have remained higher or increased in tandem (Vaast *et al.*, 1998) resulting in similar proportions of NH_4^+ use in ambient and warm plots.

We did not observe a notable interaction in heat and rainfall reduction treatments on the use of N forms. This was not expected, as we hypothesized that the impacts of rainfall reduction would be compounded by the addition of warming. For AM species, this could partially be explained by their AM fungi which could have increased their ability to maintain N uptake in drier soils (Ruiz-Lozano & Azcón, 1996; Birhane *et al.*, 2012). The unexpected response of EM species may in part be explained because we compared the proportion of N forms used, rather than the total N acquired. The combined impact of warming and reduced rainfall may be simply to reduce total N uptake (He & Dijkstra, 2014), rather than alter the form of N taken up. However, our data do not provide clear evidence of this as increases in leaf %N were associated with decreases in stem mass in the warm-reduced rainfall treatment (data not shown). EM species could also be associating with different fungi in the reduced rainfall plots that are less sensitive to warming, decreasing the overall sensitivity of their hosts to the combination of warming and reduced rainfall (Alvarez *et al.*, 2009 in Mohan *et al.*, 2014). Finally, as mentioned previously, the rainfall reduction treatment may not have lowered soil moisture enough to see compounding effects of the treatments.

The small effects of warming and rainfall reduction on the N source of species in our study could also depend on the environmental conditions at the site. If the study site

had been located further north, we may have seen greater differences in the soil concentrations of DON relative to inorganic N and thus less NH_4^+ used in ambient conditions. Additionally, the warming treatments may have had a larger impact on both boreal and temperate seedlings as the ambient soil temperatures in a cooler climate may have been below the optimal uptake temperature for both species' groups.

Changes in N source are associated with small changes in growth for some species

We expected that temperate and AM species would show increases in growth with changes in N form since warming may increase their ability to take up the forms that they grow best on. In contrast, we expected that boreal species would show more negative responses in growth to changes in N form as they may be outcompeted for their preferred form. Due to the relatively small changes in NH_4^+ use with warming and rainfall reduction, there were not strong patterns with changes in growth for most species. However, there were a few cases with clear trends in growth. EM boreal species showed consistent declines in NH_4^+ use at CFC and consistently lower biomass in treatment plots, which supported our hypothesis that they would do more poorly with changes in the forms of N they used as we expected they would already be using their preferred forms in ambient conditions. However, at HWRC only the warm-reduced rainfall treatment showed declines in NH_4^+ use and declines in growth. Since HWRC is the slightly cooler site, it is possible that the EM boreal species were able to compete more for N at that site, as indicated by increases in leaf %N and increased or similar growth.

The other noticeable trend in growth occurred in EM temperate species. At CFC, temperate EM species had slight increases in growth with changes in NH_4^+ use, which supported our hypothesis that changes in N use for temperate species would be associated with increased growth. In contrast, at HWRC the same species had lower biomass in all treatments and the greatest reductions in growth also had the greatest change in NH_4^+ use. The contrasting site responses for the EM temperate species could potentially be explained by differences in the form of N that they were using instead of NH_4^+. Since we are not entirely able to distinguish between DON and NO_3^- use, the reduction in NH_4^+ use could have been accompanied by increases in DON uptake at one site and NO_3^- uptake at the other. Since these forms may impact the growth of EM temperate species differently (Öhlund & Näsholm, 2001), this could have produced the contrasting responses. AM species did not show large changes in NH_4^+ use or leaf %N, so their increases in biomass on some of the treatments were not particularly related to any changes in N use. This may indicate that the type of N they use has little impact on their growth, as in some species (Zerihun *et al.*, 1998), and that other environmental conditions (e.g. temperature, total N availability, light) are more important in explaining their growth patterns.

Other factors contributing to deviation from hypotheses

Though the use of natural abundance isotopes can help us gain an integrated picture of N acquisition over time, the $\delta^{15}N$ signature of soil and plants can be influenced by other soil processes that would impact the interpretation of our results. First, since soils tend to become more enriched in ^{15}N with depth (Hobbie & Ouimette, 2009), if

species were acquiring a large amount of N at depths below 10 cm (i.e., the depth of our soil corer), the $\delta^{15}N$ of their N sources could be different than what we measured. However, for that to impact our estimates, the differences between the $\delta^{15}N$ values of each N form would have also had to change with depth. If the $\delta^{15}N$ of NH_4^+ did not enrich as quickly as the other forms, we would likely be overestimating the contribution of NH_4^+ for species taking up more N from lower in the soil profile.

Second, variation in the EM fungal species colonizing trees may impact how much N is transferred through the symbiosis (Hobbie & Hobbie, 2008). Since EM fungi tend to deplete the N they transfer to their hosts, this could influence the $\delta^{15}N$ of the N passed on to the tree (Hobbie *et al.*, 2012). Previous work at B4WarmED has shown changes in the EM community with warming (Fernandez *et al.*, 2017), so it is possible that the amount of N transferred through fungi is also changing. However, total colonization on roots did not change substantially with treatments in either *P. glauca* or *Q. rubra*, so the variation in N transferred due to changes in EM fungal species may be small relative to the total N transferred by EM fungi. Changes that did occur would either mean our estimates of NH_4^+ use were too high (if fungal N transfer was lower than expected), or too low (if fungal N transfer was higher than expected). In both cases, given the large estimates of NH_4^+ contribution to the N used by EM species and our moderate estimate of N transfer through fungi (~50%) in the mixing model, it seems unlikely that variation in fungal N transfer would result in a qualitatively different conclusion. Since AM fungi tend to have a smaller impact on the $\delta^{15}N$ of the N transferred to their hosts (Handley & Raven, 1992), changes in the amount of N coming from AM fungi is less likely to impact the $\delta^{15}N$ of the two AM species.

Implications and conclusions

Our study provides insight into how species use different N resources in the field, providing an integrated metric rather than an instantaneous measure of N uptake which may not capture how species partition N through time. We show that species vary in their use of different N forms, primarily according to their mycorrhizal association, with EM species using more NH_4^+ compared to AM species. This suggests that species with different mycorrhizal associations partition N by form either due to different capacities for their use, or potentially as a result of EM species being better competitors for NH_4^+ than AM species. Differences in NH_4^+ use between AM and EM species were larger than the differences between treatments, which could indicate that species have limited capacity to change N sources with climate change. However, since the changes in N availability due to warming were small it is possible that the impacts of warming on N form use would be larger in sites with greater impacts of warming on N availability. We found larger effects of reduced rainfall compared to warming, with species becoming more similar in their N use. This could indicate that species discriminate less against N forms with lower soil moisture, and that could impact species' responses to drought, as we saw that reductions in NH_4^+ use were coupled with lower plant biomass for some species. The somewhat limited capacity of species to alter their use of N forms with both warming and rainfall manipulations may result in species acquiring less total N as they may not be able to take advantage of increases in the availability of different N forms. Therefore, changes in the types of N available to plants may in part influence species responses to warming and changes in precipitation, as the species that are the best competitors for N may change altering the future composition of the ecosystem.

Table 3.1. Hypotheses for the impacts of warming and rainfall reduction on the N source and growth for different species groups. For each group, the N sources are ranked according to how much they contribute to a species' N uptake relative to the other forms. Growth responses are recorded as +/0/- to indicate changes in growth relative to ambient conditions.

Response	**Heat Treatment**	**Precipitation Treatment**	**AM**	**EM - Temperate**	**EM - Boreal**
N Source	Ambient	Ambient	NO3 = NH4 > DON	NH4 > DON > NO3	DON > NH4 > NO3
		Dry	NH4 = DON > NO3	DON = NH4 > NO3	DON > NH4 >> NO3
	+3.4C	Ambient	NO3 >> NH4 > DON	NH4 = NO3 > DON	DON > NH4 = NO3
		Dry	NO3 = NH4 > DON	NH4 = DON > NO3	DON = NH4 > NO3
Growth	Ambient	Ambient			
		Dry	0	-	-
	+3.4C	Ambient	+	+	-
		Dry	0	0/-	- - -

Table 3.2. List of species sampled in the experiment and their relevant traits.

Species Name	Abbreviation	MF type	Range	Phy
Abies balsamea	ABIBA	EM	Boreal	Gym
Acer rubrum	ACERU	AM	Temperate	Ang
Acer saccharum	ACESA	AM	Temperate	Ang
Betula papyrifera	BETPA	EM	Boreal	Ang
Picea glauca	PICGL	EM	Boreal	Gym
Pinus banksiana	PINBA	EM	Boreal	Gym
Pinus strobus	PINST	EM	Temperate	Gym
Quercus macrocarpa	QUEMA	EM	Temperate	Ang
Quercus rubra	QUERU	EM	Temperate	Ang

MF Type = mycorrhizal type; EM = ectomycorrhizal; AM = arbuscular mycorrhizal; Gym = gymnosperm; Ang = angiosperm

Table 3.3. Probability that each N form contributes more to a species' N source than the remaining forms at CFC and HWRC. High probabilities indicate that N form contributed more than either other form to a species' N uptake. Highlighting indicates probabilities greater than 0.9.

	DON				**NH4**				**NO3**			
Heat	Amb		Warm		Amb		Warm		Amb		Warm	
Water	Amb	Dry	Amb	Dry	Amb	Dry	Amb	Dry	Amb	Dry	Amb	Dry
Species												
CFC												
A. balsamea	0.007	0.078	0.023	0.109	0.980	0.811	0.939	0.791	0.013	0.110	0.039	0.100
A. rubrum	0.108	0.061	0.186	0.384	0.460	0.610	0.486	0.271	0.432	0.328	0.329	0.345
A. saccharum	0.094	0.051	0.168	0.374	0.482	0.659	0.510	0.203	0.425	0.290	0.323	0.424
B. papyrifera	0.030	0.060	0.022	0.110	0.928	0.855	0.938	0.801	0.042	0.086	0.040	0.089
P. glauca	0.060	0.070	0.013	0.045	0.861	0.822	0.950	0.919	0.079	0.108	0.037	0.036
P. banksiana	0.041	0.011	0.055	0.118	0.906	0.969	0.852	0.799	0.054	0.020	0.094	0.083
P. strobus	0.168	0.209	0.086	0.058	0.643	0.545	0.782	0.894	0.189	0.246	0.133	0.048
Q. macrocarpa	0.037	0.060	0.015	0.123	0.910	0.838	0.943	0.778	0.053	0.102	0.042	0.100
Q. rubra	0.003	0.060	0.051	0.116	0.989	0.844	0.857	0.792	0.008	0.096	0.092	0.092
HWRC												
A. balsamea	0.066	0.113	0.033	0.294	0.853	0.627	0.948	0.426	0.082	0.260	0.019	0.281
A. rubrum	0.319	0.181	0.295	0.461	0.418	0.353	0.504	0.359	0.263	0.467	0.202	0.180
A. saccharum	0.377	0.167	0.270	0.341	0.366	0.398	0.554	0.508	0.257	0.435	0.176	0.151
B. papyrifera	0.112	0.061	0.156	0.296	0.743	0.668	0.752	0.463	0.144	0.271	0.092	0.241
P. glauca	0.191	0.205	0.240	0.236	0.607	0.516	0.565	0.566	0.201	0.279	0.196	0.198
P. banksiana	0.079	0.056	0.034	0.233	0.811	0.691	0.942	0.599	0.110	0.253	0.024	0.169
P. strobus	0.003	0.115	0.014	0.241	0.993	0.613	0.981	0.587	0.004	0.272	0.005	0.173
Q. macrocarpa	0.079	0.119	0.072	0.249	0.814	0.609	0.886	0.586	0.107	0.272	0.042	0.165
Q. rubra	0.070	0.055	0.129	0.253	0.852	0.672	0.798	0.564	0.079	0.273	0.073	0.184

Table 3.4. Comparison of models explaining variation in the proportion N derived from NH_4^+. Comparisons are for a model with heat and water treatments but no additional plant traits, as well as models with different plant traits (MF type or phylogeny and range limit).

Model	**AIC**	**BIC**	$\mathbf{R^2}$	$\mathbf{R^2_{Adj}}$	**RMSE**
Site + Water + Heat	-100.89	-89.51	0.24	0.2	0.11
Site + Water + Heat + MF + Range + Range:Site + MF * Water + Site * Water	-185.2	-162.4	0.79	0.77	0.06

Table 3.5. ANOVA table for model comparing the proportion of N derived from NH_4^+ by heat and water treatments and mycorrhizal type.

Predictor	Sum Sq	Df	F value	Pr(>F)
(Intercept)	0.890	1	229.81	< 0.0001
MF	0.327	1	84.35	< 0.0001
Range	0.034	1	8.75	0.004
Site	0.085	1	21.97	< 0.0001
Heat	0.026	1	6.77	0.012
Water	0.001	1	0.42	0.52
Range * Site	0.058	1	14.91	0.0003
MF * Water	0.018	1	4.70	0.034
Site * Water	0.021	1	5.40	0.023

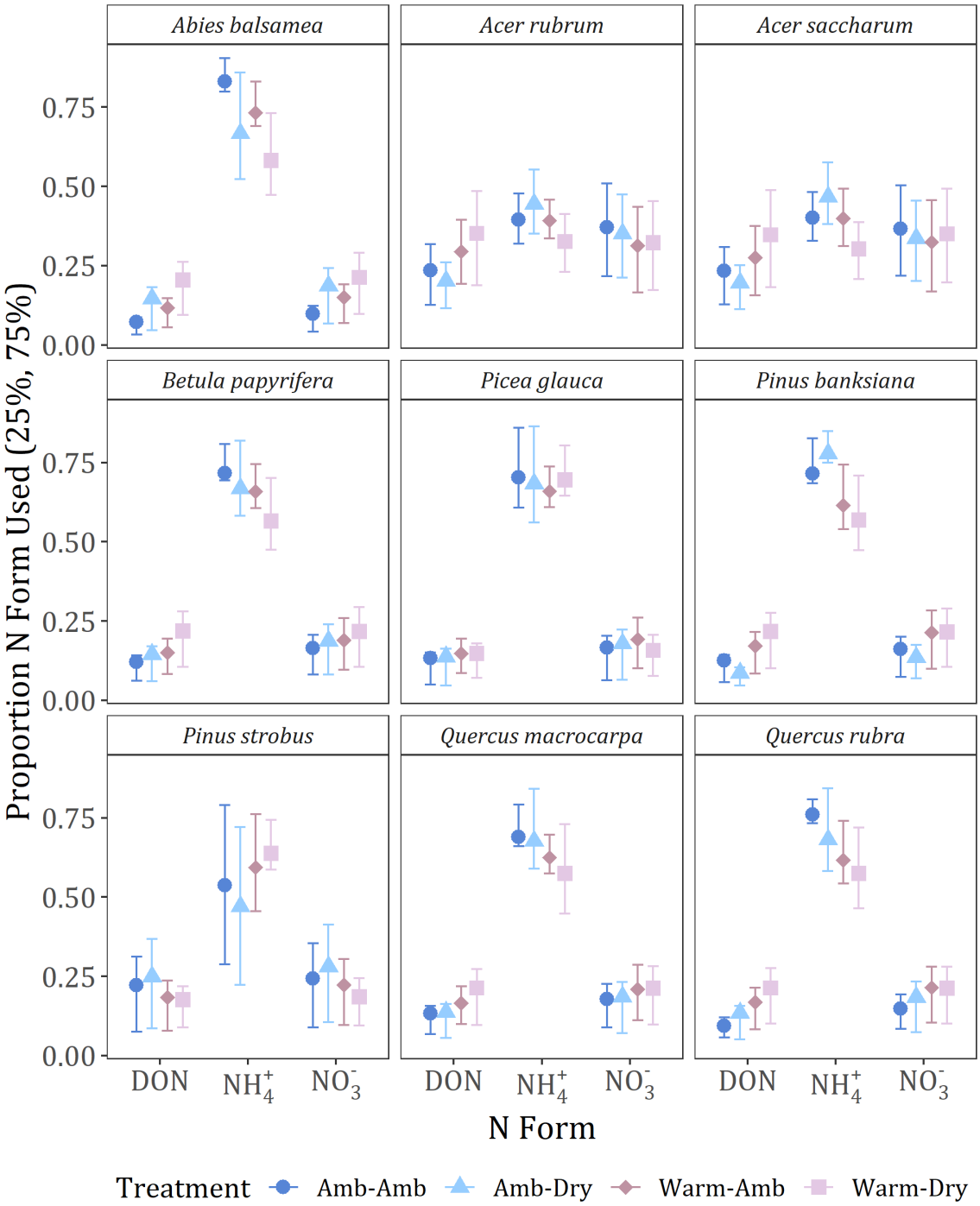

Figure 3.1. Estimated proportion of N coming from different N forms by species and treatment at CFC. Error bars represent the upper and lower quartiles of estimates from the stable isotope mixing model. All species are EM except the two *Acer* spp.

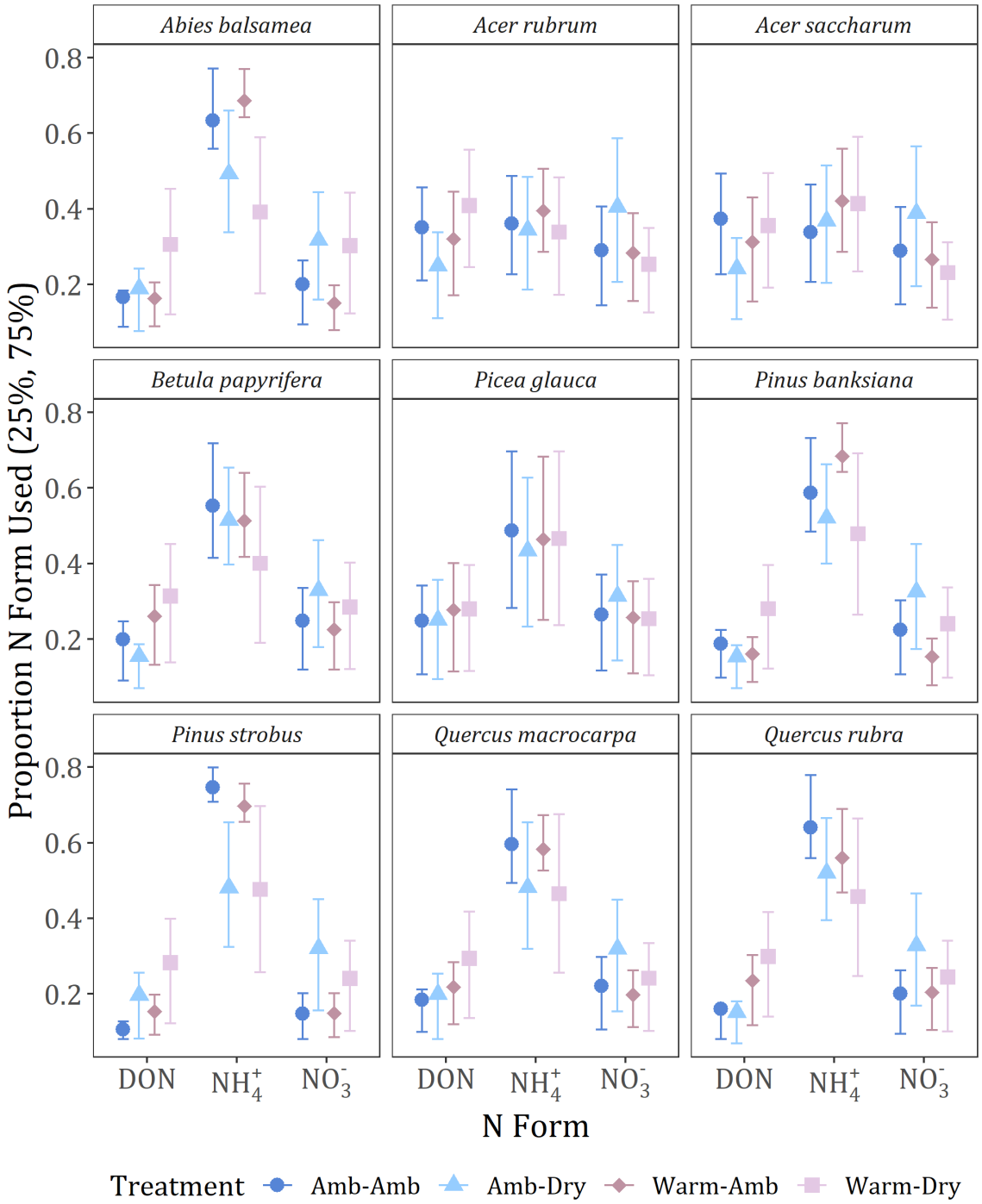

Figure 3.2. Estimated proportion of N coming from different N forms by species and treatment at HWRC. Error bars represent the upper and lower quartiles of estimates from the stable isotope mixing model. All species are EM except the two *Acer* spp.

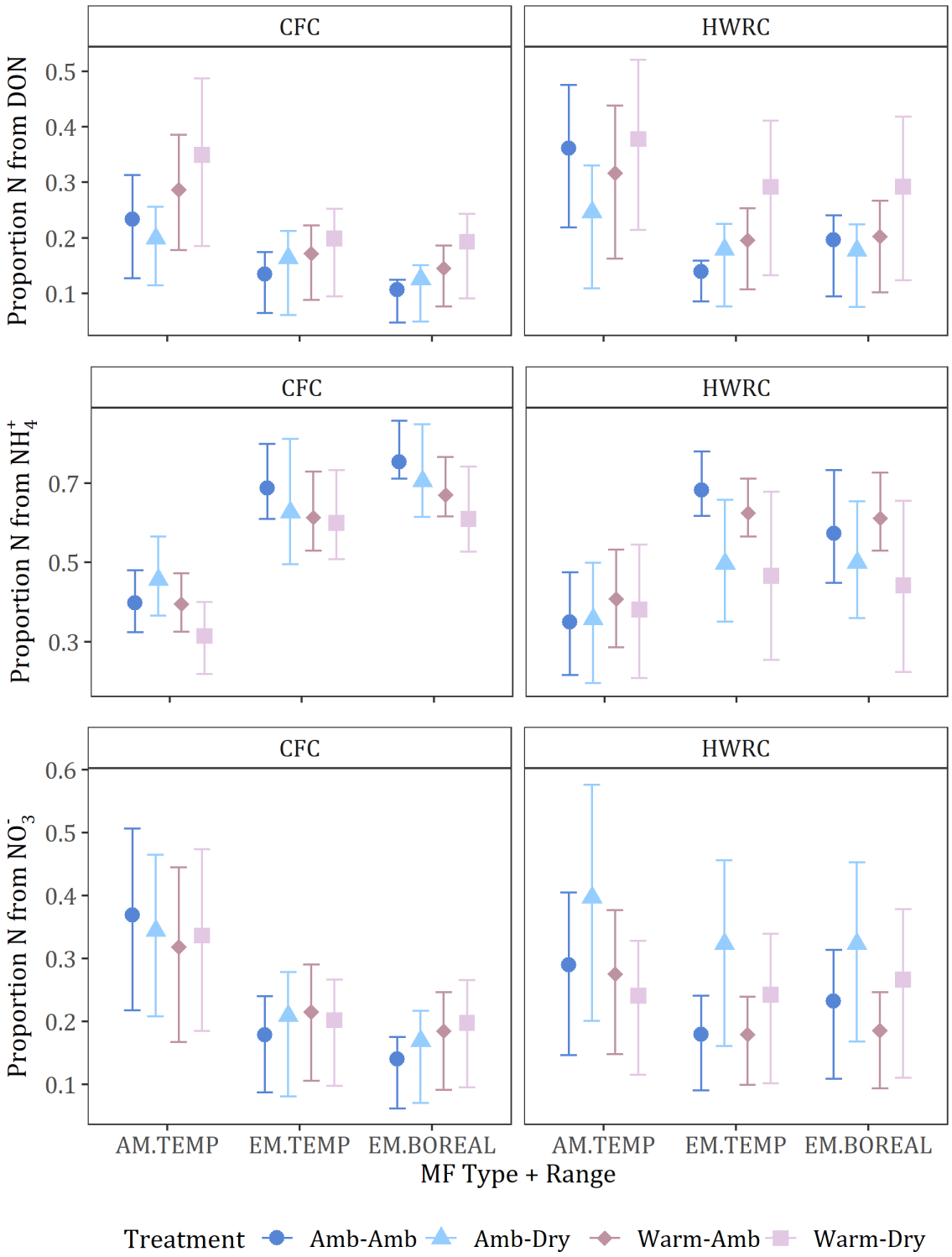

Figure 3.3. Estimated proportion of N coming from each N source by mycorrhizal type and range for each treatment. Error bars represent the 25% and 75% of estimates for each treatment, species, and site combination.

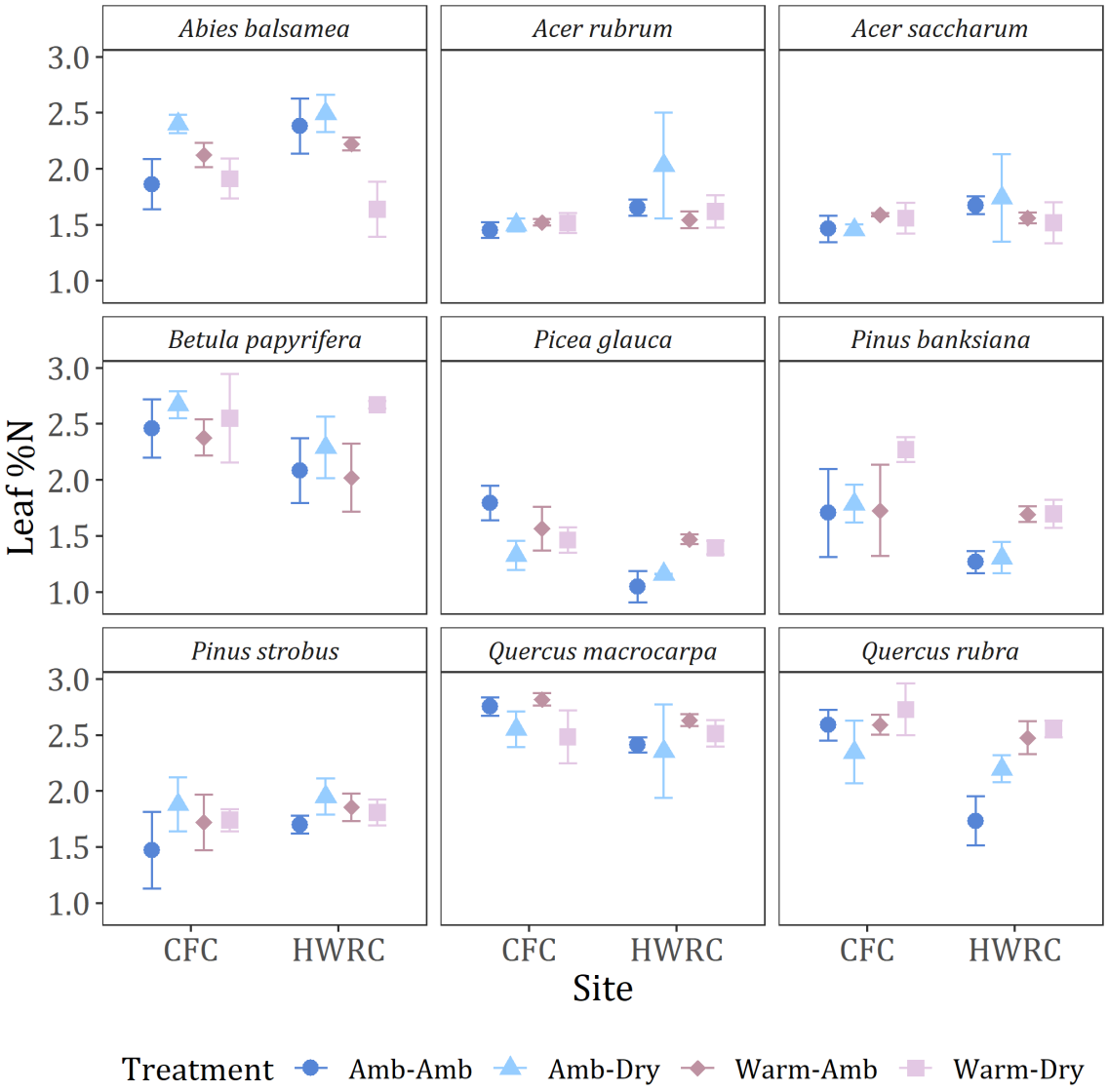

Figure 3.4. Leaf N (%) by treatment and species at each site.

CHAPTER 4

Phylogeny and mycorrhizal type are both important for explaining differences in the nutrient economies of 14 tree species in a common garden experiment

Summary

Plant species can significantly impact both carbon (C) and nutrient cycles in soils, yet their effects can be challenging to incorporate into global earth system and biogeochemical cycle models. The mycorrhizal-associated nutrient economy (MANE) is a recently proposed framework that could provide a tractable way to incorporate species effects on the C cycle into larger models. The MANE framework groups species by their mycorrhizal association (arbuscular mycorrhizal (AM) or ectomycorrhizal (EM) fungi), and this grouping has been shown to explain patterns in rates of soil nitrogen (N) cycling and the coupling between C and nutrient dynamics along gradients of EM basal area in forests. However, broad-scale phylogenetic variation within EM species in traits linked to the MANE framework suggests that there may be distinct nutrient dynamics within EM-dominated forests. However, the extent to which phylogenetic differences (e.g. angiosperm versus gymnosperm) compare to mycorrhizal differences in influencing C and N dynamics is not well understood. Furthermore, results evaluating MANE and phylogenetic frameworks have primarily come from naturally regenerating stands in temperate forests, which makes the causal nature of any associations difficult to discern. Therefore, we asked whether differences in C and nutrient cycling predicted by the MANE framework were evident in a common garden with AM and EM species, as well as whether EM angiosperms and gymnosperms had distinct C and N dynamics. We

hypothesized that we would see patterns in C and nutrient dynamics in the common garden often observed in natural stands, i.e., that AM and EM species would show distinct C and N cycling. We also hypothesized that EM gymnosperms would have C and nutrient dynamics that differ significantly from EM and AM angiosperms because of their unique traits, warranting their own category in the MANE framework.

To examine this question, we analyzed data on C and N cycling from a common garden experiment in Siemianice, Poland. This experiment consists of replicated 20m x 20m monocultures of trees growing for 30+ years and includes 14 tree species: 6 EM gymnosperms, 6 EM angiosperms, and 2 AM angiosperms. Our results partially supported our hypotheses. In some cases, metrics of C and N cycling (soil solution organic:inorganic N ratios, fine root decomposition rates) differed primarily by phylogeny. However, for other metrics, phylogeny did not matter and mycorrhizal type was important in distinguishing tree species effects: AM species had higher rates of both net N mineralization and net nitrification, while there was no significant difference by phylogeny within EM species, and litter decomposition rates primarily differed by mycorrhizal type. Overall, both mycorrhizal type and phylogeny served as important predictors for C and N cycling.

Introduction

The increasing rate of anthropogenic CO_2 emissions, the rise of atmospheric CO_2 concentrations, and the role of ecosystem feedbacks on that rise accentuate the need for accurate models of the global carbon (C) cycle to better predict the extent and potential consequences of climate change. Models that lack the proper controls on key processes in the C cycle may result in inaccurate projections for atmospheric CO_2, especially when extrapolating beyond our current conditions. Terrestrial ecosystem processes, such as decomposition and net primary production (NPP), are responsible for C fluxes around ten times greater in magnitude than anthropogenic emissions (Friedlingstein *et al.*, 2010, 2019; Zhang *et al.*, 2017). Despite the importance of these processes to the C cycle, it remains a challenge to accurately incorporate them into models. For example, nutrient limitation was frequently omitted from early C cycle models (Zaehle *et al.*, 2015; Thomas *et al.*, 2015), and is still overly simplistic in most models (Brzostek *et al.*, 2014), which can result in overestimation of NPP responses to elevated CO_2 (Reich *et al.*, 2006; Thomas *et al.*, 2015). Since a large amount of C is invested in nutrient acquisition (Shi *et al.*, 2016), improving our understanding of how C and nutrient cycles are linked will improve estimates of how much C is allocated to NPP versus plant respiration.

One major challenge to improving our synthetic understanding involves the effects of species on C cycle processes and how those may differ in their response to climate change and elevated CO_2. Numerous studies highlight the importance of considering species identity or plant functional traits when evaluating controls on ecosystem processes (Diaz & Cabido, 2001; Cornwell *et al.*, 2008b; Pastore *et al.*, 2020).

For example, plant biomass production in response to elevated CO_2 varied by mycorrhizal type, with species that associated with ectomycorrhizal fungi (EM) showing a 30% increase in biomass in response to elevated CO_2 while AM species showed no response (Terrer *et al.*, 2016). Until recently, it was most common to group tree species into functional groups based on their leaf lifespan (deciduous versus evergreen) and phylogeny (angiosperms versus gymnosperms) to compare their impacts on ecosystem processes (Reich *et al.*, 2007; Mueller *et al.*, 2012b; Augusto *et al.*, 2015). While this captures a decent amount of variation in tree species effects on processes involved in C and N cycling (Reich *et al.*, 1997; Cornwell *et al.*, 2008b), there remains a large amount of unexplained variation within these categories. For example, oaks (*Quercus* spp.) and maples (*Acer* spp.) are both deciduous angiosperms but vary significantly in the rates of decomposition of their leaf litter (Hobbie, 2008) and in their rates of N cycling (Venterea *et al.*, 2003; Lovett *et al.*, 2004; Weand *et al.*, 2010). As forests cover over 28% of land surface area (Bartholomé & Belward, 2005), and large amounts of forested land are covered by trees of similar leaf lifespans and/or phylogenetic grouping, researchers have proposed other groupings to help explain some of this variation.

One such grouping that has received considerable attention is the mycorrhizal-associated nutrient economy, or MANE, framework proposed by Phillips *et al.* (2013). The MANE framework proposes grouping species by the type of mycorrhizal fungi that they associate with—arbuscular mycorrhizal (AM) fungi or ectomycorrhizal (EM) fungi. The initial support provided for this grouping came from studies documenting differences in rates of C and N cycling in forest stands dominated by AM or EM species. Trees that associate with AM fungi were found to have higher quality leaf litter that decomposed at

faster rates than EM litter (Phillips *et al.*, 2013), which promotes greater rates of N mineralization and nitrification. EM species tended to have lower quality leaf litter and rely on N- and P-mining enzymes provided by their fungal partners to help acquire N and P tied up in the slowly decomposing leaf litter. Essentially, plots dominated by AM species should have an "inorganic nutrient economy" versus an "organic nutrient economy" in plots where EM species dominate. Major strengths of this framework include its ability to predict ecosystem responses to global change factors (Midgley & Phillips, 2014; Terrer *et al.*, 2016).

Since its publication, a number of studies have shown support for the MANE framework and demonstrated its usefulness in predicting ecosystem responses to global change (Midgley & Phillips, 2014; Corrales *et al.*, 2016; Cheeke *et al.*, 2017; Lin *et al.*, 2018). For example, studies of leaf litter decomposition have generally supported the framework, with leaf litter from AM trees decomposing more quickly than that from EM trees (Midgley *et al.*, 2015; Jacobs *et al.*, 2018; Keller & Phillips, 2019), though not in all systems (Keller & Phillips, 2019). Further support for the framework comes from a study in Chinese temperate forests that found significantly lower rates of N cycling (net N mineralization and net nitrification) and lower NH_4^+ and NO_3^- concentrations in EM stands compared to AM stands, which the authors attributed to the lower decomposability of EM relative to AM litter (Lin *et al.*, 2018). An increasing number of studies are now exploring additional processes and mechanisms underlying this framework, such as differences between mycorrhizal types in root exudation and rhizosphere priming (Sulman *et al.*, 2017; Liese *et al.*, 2018), plant traits (Averill *et al.*, 2019), microbial community composition and function (Taylor *et al.*, 2016; Wurzburger *et al.*, 2017;

Cheeke *et al.*, 2017), soil C dynamics (Verbruggen *et al.*, 2016b,a; Frey, 2019), and ecosystem responses to global change (Mccormack *et al.*, 2014; Mohan *et al.*, 2014). These studies offer significant support for this framework and emphasize its potential usefulness in understanding future impacts of global change on ecosystem processes.

Despite the growing body of research, there remain several untested questions about the applicability of this framework. For one, most studies have occurred in natural forest stands rather than in experiments (Table S4.1), so the extent to which EM or AM species actively cultivate these nutrient economies remains an open question. For example, AM species could simply outperform EM species on soils that tend to be dominated by inorganic nutrients, rather than increasing the rate of nutrient cycling and availability of inorganic nutrients in places where they are more abundant. In fact, there may be many other factors that are not controlled for in a significant portion of these studies, including soil type (age, texture, parent material), stand age (Blaško *et al.*, 2015), soil organisms, soil moisture (Schilling *et al.*, 2016), and temperature. Since some studies have found substantial variation in N cycling within a single forest plot (Waring *et al.*, 2016), controlling for as many of the aforementioned factors as possible will provide the strongest tests of this framework.

Additionally, the initial framework did not consider differences between angiosperms and gymnosperms within mycorrhizal type, even though litter quality and decomposition (a driving factor in the MANE framework) frequently differ(s) between the two types of species (Hobbie *et al.*, 2006; Reich *et al.*, 2007; Cornwell *et al.*, 2008b). Root traits have also been shown to differ substantially by phylogeny (Comas &

Eissenstat, 2009; Wang *et al.*, 2018; Yahara *et al.*, 2019), which could play a role in creating a distinct nutrient economy in gymnosperm-dominated stands as root inputs can be a substantial fraction of C inputs to ecosystems – up to 22% of terrestrial NPP by some estimates (McCormack *et al.*, 2015). Root traits were also not directly considered in the original description of the MANE framework (but see Jacobs *et al.* (2018)). Of the studies that do include gymnosperms in comparisons of soil C and N dynamics, many are primarily in N. American forests or represent limited variation across other factors (e.g. soil age/parent material). Since large portions of the globe are covered by EM forests with large C stocks where angiosperms and gymnosperms co-occur (e.g. boreal and temperate forests (Bradshaw *et al.*, 2009; Bradshaw & Warkentin, 2015)), testing whether EM angiosperms and gymnosperms have similar or distinct nutrient economies is important.

To improve the usefulness of the MANE framework to global C models, our study aims to address the following knowledge gaps. First, we asked whether distinct nutrient economies developed under AM and EM species in common-garden forest stands with the same initial conditions. Second, we asked whether stands dominated by EM gymnosperms had the same nutrient economy as those dominated by EM angiosperms. Finally, we asked how root traits (chemical quality, decomposition rates, and root inputs) contributed to the nutrient economies of AM versus EM species and angiosperms versus gymnosperms. We hypothesized that (1) we would see distinct nutrient economies in AM compared to EM dominated stands due to the influence of plant traits and their associated microbial communities on key C and N cycle processes; (2) EM gymnosperms would be an extension of the MANE framework, with a more

extreme organic nutrient economy than that of EM angiosperms, caused by lower decomposition rates from lower quality and more acidifying litter; and (3) root traits would play an important role in differentiating the nutrient economies of both AM and EM associated species as well as angiosperms and gymnosperms, as previous studies have found differences in nutrient foraging and morphological traits by mycorrhizal type (Comas *et al.*, 2014; Chen *et al.*, 2016) and phylogeny (Comas & Eissenstat, 2009).

To examine these questions, we use data from a common garden experiment with 14 tree species established in Siemianice, Poland (Reich *et al.*, 2005). This experiment has data collected on both C and N cycling in monocultures of trees growing for 30+ years. Utilizing the common garden approach enables us to control for many factors, such as differences in parent material, stand age, climate, and topographic position, which allows us to more clearly isolate species effects on these processes. While some of these data have been reported in previous publications, those have primarily focused on taking a more mechanistic approach to understand how plant traits impact specific processes (e.g. leaf litter and fine root decomposition: Hobbie *et al.* (2006, 2010)) or nutrient cycles (e.g. soil C and N cycles (Hobbie *et al.*, 2007; Mueller *et al.*, 2012a)). None of the data have been analyzed with the purpose of comparing C and N dynamics in the context of the MANE framework, so while previous studies have identified important links between plant traits and ecosystem processes, the data have not been used to examine whether larger trait groupings (e.g. mycorrhizal type and phylogeny) have distinct patterns of C and N cycles. Additionally, the common garden includes an incomplete factorial design/test of mycorrhizal type and phylogeny with six EM angiosperms, six EM gymnosperms, and two AM angiosperms which allows us to examine how patterns in C

and N cycles develop as a result of both mycorrhizal association and phylogeny starting from the same conditions, although we are unable to compare AM angiosperms and gymnosperms in this design. These data and comparisons will improve our understanding of how species influence the patterns documented in the MANE framework, as well as whether additional trait groupings (e.g. phylogeny) expand the utility of the framework. Both tests will allow for better incorporation of plant functional groups into global C models to improve predictions of C cycling in the future.

Methods

Study location

The data used in this analysis come from a common garden experiment (Reich *et al.*, 2005) established in the Siemianice Experimental Forest near Biadaszki, Poland (51°14.97'N, 18°06.35'E, elevation 150m). The site has a mean annual temperature of 8.2 °C, with a mean annual precipitation of 591 mm (Reich *et al.*, 2005). The common garden consists of 14 tree species planted in 20m x 20m monoculture plots and was established in 1970 and 1971. Prior to the experiment, the study area was an 81-year old Scots Pine (*Pinus sylvestris* L.) stand, which was harvested, and then soil was tilled prior to planting. Trees were planted at 1m x 1m spacing, and included 2 AM angiosperms, 6 EM gymnosperms, and 6 EM angiosperms, with both native European and non-native N. American species (Table 4.1). Plots were established in two blocks, with 9 species replicated 3 times in each, and 4 species (*Larix decidua*, *Picea abies*, *Pseudotsuga menziesii*, *Quercus robur*) planted in both blocks for a total of 54 plots. One of the plots

for *Abies alba* was not usable, so the analysis only used 53 plots. See Reich *et al.* (2005) for additional details on experimental design and initial sampling. Here we report measurements that were made between 2002-2004, except where noted.

Leaf litter and fine root chemistry

We compiled data on both above- and belowground plant traits likely to influence C and N dynamics. These include previously published leaf litter and fine root (< 2mm diameter) lignin content, %N, %C, C:N ratio, lignin:N ratio, P content, and calcium content (Hobbie *et al.*, 2006, 2010). Data on leaf litter traits were collected from litter collected in litterfall traps between 31 May 1996 and 30 Nov 1998 (Hobbie *et al.*, 2006). Root trait data were collected from fresh roots sampled in 2002 using a root corer with 8 cores per plot to a depth of 15 cm (Hobbie *et al.*, 2010). Roots were sieved to select roots < 2mm in diameter and dried at 65°C before analysis. See Hobbie *et al.* (2006) and Hobbie *et al.* (2010) for more detailed descriptions of leaf litter and fine root sampling and processing.

Soil carbon dynamics

To examine the influence of mycorrhizal type and phylogeny on soil carbon cycling, we assembled published and unpublished data on numerous pools and fluxes of C from the common garden plots. We looked at the inputs of leaf litter using published litterfall estimates (Hobbie *et al.*, 2006). Fine root inputs were estimated using root

ingrowth cores placed in plots and collected after 1 year. The ingrowth cores were constructed using plastic screen rolled into a tube 3 cm in diameter and 25 cm long, with 1.5 x 1.5 mm holes. The cores were installed in plots vertically in 3 random locations and filled with sieved, root-free mineral soil, and were left for 1-year before removing. Roots were separated into fine roots (< 2 mm diameter) and coarse roots (> 2 mm diameter), dried at 50 °C for 48 hrs, and weighed. We then calculated the annual biomass inputs to plots as well as the proportion of tree biomass inputs to plots coming from litter vs. roots, as these have different implications for soil C storage.

We examined leaf litter and fine root decomposition from previously published litter bag and forest floor mass balance studies. Leaf litter decay rates came from a published decomposition experiment of litter decomposing in its plot of origin (the "Home Plot" experiment in Hobbie *et al.*, 2006). Forest floor turnover rates also come from (Hobbie *et al.*, 2006). Fine root decay rates were obtained from a published fine root decomposition experiment with 11 species (2 AM angiosperms, 5 EM angiosperms, and 4 EM gymnosperms; *Betula pendula* and the two *Pinus* spp. were not included) conducted in a common plot populated by *Acer pseudoplantanus* (Hobbie *et al.*, 2010).

Soil nitrogen dynamics

Rates of net N mineralization and net nitrification were obtained from published data of both laboratory and *in situ* incubations (Hobbie *et al.*, 2007). Laboratory incubations for potential N mineralization and nitrification rates included both short (30-day) and long-term (385-day) incubations of soil samples from the O and A horizons

(Hobbie *et al.*, 2007). Potential N mineralization and nitrification rates are presented from the short-term incubations, while data from the long-term incubations were used to determine total dissolved nitrogen (TDN) and total dissolved organic carbon (DOC). Total dissolved organic nitrogen (DON) was calculated by subtracting the average DIN concentration from the *in situ* incubations from the TDN and calculate the ratio of DON:DIN. *In situ* rates of N mineralization and nitrification were measured over one year (5-week intervals from May thru October, and one long overwinter incubation) using the capped-core method (Hobbie *et al.*, 2007). Rates were summed to determine the annual net N mineralization and nitrification rates per unit area.

We also examined ion-exchange resin bag estimates of N availability (also presented in Mueller *et al.*, 2012a). Three pairs of ion-exchange resin (IER) bags were installed randomly in each plot in both the O and A horizons. Resin bags were deployed in mid-May and were sampled and replaced at ~5 week intervals thru mid-October. Each bag was frozen upon collection until extraction and analysis at the University of Minnesota. For each plot, the accumulation of N was averaged across the three resin bags for each depth and then summed across the three sampling periods to determine the IER-N accumulation during the growing season.

Soil C and N by depth

We calculated whole-profile soil C and N using soil bulk density data and soil % C and %N data from soil pits. The sampling is described in more detail in Reich *et al.* (2005) and Hobbie *et al.* (2007) along with the published data from the top 0.40 m, but

here we present data down to 0.75 m from soil pits dug in each plot (down to the C horizon, parent material). Samples were taken from each soil horizon, and we used the bulk density and %C and %N to determine the total mass of C and N in each horizon and summed the horizons to calculate total C, N, and the C:N ratio. We also separated out the total C, N, and C:N by O horizon, the top 10 cm of mineral soil, and all mineral soil (to 0.75m) to compare whether the major differences in C and N storage varied by organic versus mineral soil and by depth of the mineral soil. We also calculated the proportion of C in the top 10cm of soil (with or without the O horizon) to see if our patterns of soil C and N distribution differ by MF type, similar to findings in Craig *et al.* (2018).

Microbial community data

We combined published and unpublished data on the microbial community composition and pools of C and N to examine differences by mycorrhizal type and phylogeny. Microbial biomass C and N were determined by the chloroform fumigation method (Brookes *et al.*, 1985) and published in Hobbie *et al.* (2007). While there is no direct measure of fungal biomass, we report a few different metrics describing the fungal community. The abundance of fungi and bacteria were measured using PLFA analysis (Frostegård *et al.*, 1993). Additionally, we use EM species richness (from root tips and sporocarps) as an indicator of the EM fungal influence. Although we did not have direct measures of mycorrhizal fungal abundance, we assumed that the dominant mycorrhizal fungal type (EM versus AM) in each plot reflected that of the overstory tree species, since the high density of planted trees reduced understory plant growth in the plots.

Edaphic and environmental variables

Other potentially important covariates relating to soil properties and soil fauna were measured between 1995 – 2002, of which some are published (Reich *et al.*, 2005). Soil pH, earthworm abundance and biomass, % clay, % base saturation and CEC for the top 40cm of soil were reported in Reich *et al.* (2005) and (Hobbie *et al.*, 2007) and measured with traditional methods (SSL Staff, 2004) except for earthworm abundance which was measured using mustard extractions (Hale *et al.*, 2005). Data on mean soil temperature and soil moisture were also analyzed as potential covariates in some analyses, and were reported in (Hobbie *et al.*, 2007).

Calculations and statistical analyses

All statistical analyses were conducted in R version 4.0.0 (R Core Team, 2020). We checked for outliers in the data using the *scores* function from the *outliers* package v. 0.14 (Komsta, L. 2011). Additional details are provided in Appendix 4 in the supporting methods section.

We tested whether mycorrhizal type, phylogeny, or both were important predictors for characteristics and processes relevant to the MANE framework using a combination of linear models (LM) and linear mixed effects models (LMM) following the protocol in Zuur *et al.* (2009). For all model comparisons, we selected the best model by comparing their AIC and BIC values generated by the *anova* function (*stats* package

v. 4.0.0, R Core Team 2020), with a ΔAIC or ΔBIC value > 2 used as the cutoff for determining the best model. First, for each dependent variable we tested whether a random effect was needed by fitting a linear model with mycorrhizal type and phylogeny as predictors and comparing it to a LMM with the same predictors and with species as a random effect (linear model fit with the *gls* function and LMM fit with the *lme* function, both functions from the *nlme* package v. 3.1-147, Pinheiro *et al.* 2020). In cases where a random effect did not result in a better fit, we followed the next steps for both LM and LMM to see if results differed qualitatively, and if they did not, we present LMM results for consistency. We then determined the best fixed effects structure by comparing the model with both mycorrhizal type and phylogeny to ones with just mycorrhizal type or just phylogeny, using the maximum likelihood method for LMM. After comparing the three models to determine the best fit, we refit the best model, if using LMM, with the restricted maximum likelihood estimation (REML) and present the model summary and post-hoc comparisons using the *summary* and *emmeans* (package *emmeans* v. 1.4.6, Lenth et al. 2020) functions. In the case where some or all models were comparable (AIC and BIC values within 2), we report the model summary for the model with the lowest AIC value. To evaluate the models' explanatory power, we present the conditional R^2 (R^2_{cond}) to summarize the variation explained by both the fixed and random effects, and the marginal R^2 (R^2_{marg}) which summarizes the variation explained by just the fixed effects (Nakagawa & Schielzeth, 2013).

For the analysis of variables relating to soil properties or processes, we followed the same protocol above but also tested additional covariates that may influence these processes when the R^2_{marg} was exceptionally low (< 0.10) or contrary to our expectations.

We looked for correlations between the variable of interest and other soil properties thought to impact the process, including soil moisture, temperature, soil texture, and the microbial community. We used the *cor* function from the *stats* package, and for variables that were significantly ($p < 0.05$) and strongly (pearson's $|r| > 0.4$) correlated we compared LMMs with and without the different covariates. If the covariates added significant explanatory power to the model, we also report those results.

For all models, we used the *performance* package (v. 0.4.7) to test compliance with model assumptions. The *check_model* function produced plots to test for normality, homogeneity of variance, homoscedasticity, and where applicable, the normality of random effects. To use this function, we refit the best model with the *lmer* function (*lme4* package) as the package works best with that function and the model estimates are similar. In mild cases, we did not perform additional corrections as the models have been shown to be robust in many cases (Schielzeth *et al.*, 2020). More concerning cases tended to be models with low explanatory power ($R^2_{cond} < 0.1$) so these results have little impact on our overall conclusions.

Finally, we conducted a principal component analysis (PCA) to determine whether any differences in C and N metrics resulted in distinct groups by mycorrhizal type, phylogeny, or both. We selected variables related to C and N pools and fluxes, rather than soil properties, and included separate variables where applicable for pools and fluxes from the O vs. A horizons. We ran the PCA on centered and standardized variables to account for the differences in units and scales of each metric. The PCA was conducted with the *prcomp* function (*stats* package), and correlations and contributions to the PCA

axes were determined using the *get_pca_var* function (*factoextra* package v. 1.0.7). We checked that our data were well suited for a PCA by confirming that we could explain at least 70-80% of the variation with principle components that had eigenvalues > 1 (meaning that they add more explanatory power than a single variable since we standardized the data) (Nistrup, 2019).

Results

Leaf litter and fine root decomposition rates

Both mycorrhizal type and phylogeny were important in explaining variation in leaf litter decomposition and forest floor turnover. AM angiosperms had the highest rates of leaf litter decomposition and forest floor turnover, though these metrics differed in the magnitude and significance of the effects. All models for the Home Plot leaf litter decomposition rates had AIC and BIC values within 3, though the model with only mycorrhizal type had a slightly lower BIC value than the other two models (Table S4.2). However, the random effect (species) accounted for more variation in the data than the fixed effects (Table 4.2). Decomposition rates were slightly higher for AM angiosperms compared to both EM angiosperms and EM gymnosperms (Figure 4.1), though the 95% CI overlapped significantly (Table 4.3). We observed the same pattern in forest floor turnover, though the magnitude of the effects was much greater. The model with both MF type and phylogeny had much lower AIC and BIC values than each group alone and explained a large amount of variation in the data ($R^2_{cond} = 0.62$, $R^2_{marg} = 0.46$). AM-dominated stands had forest floor turnover rates ~2.9X greater than EM angiosperms (p =

0.030) and ~11.5X greater than EM gymnosperms ($p = 0.0009$). EM angiosperms had about 4X higher turnover rates than EM gymnosperms ($p = 0.04$).

Fine root decomposition followed a similar trend to leaf litter decomposition, with the fastest decomposition rates in AM angiosperm plots and the slowest in EM gymnosperm plots. However, only phylogeny was included in the best model, though it explained a small amount of variation in decomposition rates ($R^2 = 0.14$, Table S4.2, Figure 4.1). Roots of AM and EM angiosperms decomposed ~25% more quickly roots of EM gymnosperms ($p = 0.11$). Additionally, comparing the decomposition rates of AM angiosperm (*Acer* spp.) leaf litter and fine roots shows that the fine roots decomposed more quickly than the leaf litter (Figure 4.1).

Leaf litter and fine root chemistry

For all leaf and fine root traits examined, differences in tissue chemistry were either best explained by phylogeny, not by mycorrhizal type, or traits were similar across groups (Table S4.3). Leaf litter chemistry indicated that both EM and AM angiosperms tended to have higher quality litter than EM gymnosperms, with slightly lower lignin:N ratios, lower C:N ratios, and higher phosphorus (P) concentrations (Table 4.4). However, for all leaf traits, more variation could be attributed to the influence of individual species over the fixed effect of phylogeny (Table S4.3). Root chemistry also showed strong species effects, with low R^2_{marg} vs. the R^2_{cond} (Table S4.3), but tissue quality tended to be higher in gymnosperms than in angiosperms (Tables 4.3, 4.4). The lignin:N and C:N ratios were ~ 25% and 19% lower for EM gymnosperms compared to angiosperms,

respectively ($p = 0.033$, $p = 0.06$, respectively), though root P concentrations were similar between the two groups ($p = 0.568$).

Litterfall and root production

For all species groups, annual leaf litterfall was greater than root production. This ratio was highest for EM angiosperms (leaf:root inputs = 3.7) and lowest for AM angiosperms (leaf:root inputs = 1.7). Litterfall varied most strongly by phylogeny (Table 4.2, 4.3, 4.4), though a substantial amount of variation in the model was due to species differences ($R^2_{cond} = 0.71$ vs. $R^2_{marg} = 0.23$). Litterfall was ~1.7X greater in AM and EM angiosperm plots compared to EM gymnosperm plots ($p = 0.037$). In contrast, root inputs varied primarily by mycorrhizal type (Table 4.2, 4.3, 4.4), and proportionally more variation was explained by the mycorrhizal type fixed effect compared to the species random effect (Table 4.2). AM angiosperms had 2.4X greater root inputs than EM angiosperms and gymnosperms ($p = 0.007$, Table 4.3).

Overall, total inputs (fine root ingrowth + leaf litterfall) were best explained by a model with phylogeny (Table S4.1), though AM angiosperms did tend to have higher total inputs than either EM angiosperms or EM gymnosperms (Table 4.4). AM and EM angiosperms had on average 1.5X more total plant inputs than EM gymnosperms ($p = 0.025$, Table 4.3), though the random effect also accounted for a large amount of variation in the total inputs (Table 4.2).

Nitrogen cycling

The N cycling metrics we examined differed in which model best explained variation in the data. Phylogeny explained more variation in DON:DIN ratios and inorganic N accumulation on resins, but mycorrhizal type was more important for net N mineralization and net nitrification rates. Furthermore, potential rates of N mineralization and nitrification were similar across groups.

DON:DIN ratios varied more by phylogeny than mycorrhizal type (Table S4.2), with DON:DIN ratios in both EM and AM angiosperm plots at least 2X greater than those in EM gymnosperm plots ($p = 0.001$, Figure 4.2, Table 4.2). To explore this relationship further, we examined the influence of EM fungal species richness on DON:DIN in just EM angiosperm and gymnosperm plots (Figure 4.2 inset). Adding EM fungal richness to the model improved the AIC value ($\Delta AIC = 2.64$) but slightly worsened the BIC value ($\Delta BIC = -0.8$), though it did improve the R^2 (new model: $R^2_{cond} = 0.43$, $R^2_{marg} = 0.38$, see Table 4.2 for original). As EM fungal richness increased, DON:DIN also increased significantly (slope (95% CI) = 0.115 (0.0034 – 0.226)). When accounting for EM richness, gymnosperms still had slightly lower DON:DIN ratios overall ($t = 1.76$, $p = 0.10$).

In contrast to DON:DIN ratios, rates of *in situ* net N mineralization and net nitrification both varied primarily according to mycorrhizal type rather than phylogeny. AM angiosperm plots tended to have greater rates of N cycling compared to all EM plots (Figure 4.3), however, the model fit was poor ($R^2_{Nmin} = 0.08$, $R^2_{nit} = 0.05$) and the differences were small for nitrification. Additionally, overall rates of net nitrification

were less than half the rate of net N mineralization (Figure 4.3). Given the low explanatory power, we tried to control for other variables that may influence soil N cycling. For N mineralization and nitrification, the average soil moisture explained much more variation in their rates compared with mycorrhizal type or phylogeny (Figure 4.3 inset). MF type was also no longer significant when soil moisture was included for N mineralization (Table S4.3). Though including soil moisture improved the model fit for nitrification rates, nitrification rates had a slightly stronger relationship with the fungal:bacterial ratio in a plot (R^2 = 0.47 vs. 0.4). Nitrification rates declined as fungal:bacterial ratios increased (Table S4.3), and after accounting for differences in the fungal:bacterial ratio, neither mycorrhizal type nor phylogeny were important predictors of nitrification rates (Table S4.4). AM angiosperms tended to have lower fungal:bacterial ratios (0.08 vs 0.15) which help explain their 2X higher rates of nitrification than EM angiosperms ($t = 2.5$, $p = 0.038$).

Given that N cycling metrics can be quite variable we also report potential nitrification and mineralization as well as ion-exchange resin bag DIN (NH_4^+ and NO_3^-) accumulation and examined their correlations with the *in-situ* measurements of N cycling. The accumulation of NH_4^+ and NO_3^- on resins differed predominantly by phylogeny for both N forms, with a significant interaction between phylogeny and horizon (Table S4.3, Figure S4.2). Fixed effects explained a large amount of variation, though species also made an important contribution to the model (Table 4.2). N accumulation on resins was higher in the A horizon compared to the O horizon for both NH_4^+ and NO_3^-, and this difference was largest for EM gymnosperms (Table S4.4). EM gymnosperms also had greater N accumulation on resins than AM and EM angiosperms

for both NH_4^+ and NO_3^-, though the effect was slightly larger for NH_4^+ (Table S4.4). In contrast to the resin data, potential N mineralization rates did not differ by mycorrhizal type or phylogeny, though the rates were higher in O compared to the A horizon (Tables S4.3, S4.4 and Figure S4.3). The species random effect also explained more variation in the model than the fixed effects (Table S4.3). Potential nitrification rates were less well explained by the model, but phylogeny explained a small portion of the variation in the rates, with EM gymnosperms showing slightly higher rates of potential nitrification than both AM and EM angiosperms (Table S4.3). Since the *in-situ* N cycling measurements showed different patterns from the potential N cycling metrics, exploring how they differ can offer insight into the factors that are driving variation in N availability in these plots.

Soil C and N storage

The model that best explained variation in soil C depended on the soil horizon and depth. Overall, total C storage (O horizon down to 75 cm in the mineral soil) was best predicted by phylogeny (Table 4.2), with EM gymnosperms storing ~ 1.3 kg C/m^2 more than AM and EM angiosperms ($t = -2.64$, $p = 0.023$, $df = 11.3$, Table 4.3). However, this was primarily due to the larger amount of C stored in the O horizon in EM gymnosperm plots, as AM and EM angiosperms stored similar amounts of C in the top 75 cm of mineral soil and much less in the O horizon (Figure 4.4). Total C in the O horizon was best explained by a model with both MF type and phylogeny, with EM gymnosperms storing the most C and AM angiosperms storing the least amount of C (Table 4.2, 4.3). For C stored in the O horizon, top 10cm of mineral soil, and whole profile, the species

random effect also explained at least 50% of the variation explained by the whole model (Table 4.2, S4.3), though explanatory power was low for models that included the mineral soil. We also found that the proportion of C stored in the top 10 cm of soil was best explained by a model with just mycorrhizal type, though these differences were relatively small and more variable by species (Table 4.2, 4.3 and Fig. S4.4).

Soil C:N ratios differed primarily by phylogeny when considering the whole profile, but the trends differed slightly with mycorrhizal type by depth and were primarily driven by differences in soil C, not N. Whole profile C:N ratios were best explained by phylogeny, as EM gymnosperms had higher C:N ratios than either AM or EM angiosperms, but in the top 20-30 cm both EM gymnosperms and angiosperms had similar C:N ratios (Figure S4.1). These trends were driven primarily by changes in the amount of C at each depth, as N content did not vary significantly by mycorrhizal type or phylogeny with depth (Figure S4.1).

DOC, Microbial biomass, and microbial community

We also examined several other variables related to ecosystem C cycling, including DOC, microbial biomass C, and microbial community composition. DOC in soils differed primarily by horizon rather than by mycorrhizal type and phylogeny. Concentrations of DOC were an order of magnitude higher in the O horizon compared to the A horizon (Figure 4.5), whereas differences within horizons were much smaller. EM gymnosperms had slightly greater DOC in the A horizon compared to AM or EM angiosperms ($t = -1.78$, $p = 0.10$, $df = 11.1$), while in the O horizon DOC concentrations

were similar across groups though EM angiosperms tended to have slightly higher DOC. In contrast to DOC, microbial biomass C (per g C) differed primarily by mycorrhizal type and phylogeny rather than horizon (Table S4.3). In both the O and A horizons, microbial biomass C was highest for AM angiosperms and lowest for EM gymnosperms, and on average, AM plots had about twice the amount of microbial biomass C as EM gymnosperms (Fig. S4.5). However, there was an interaction between horizon and mycorrhizal type + phylogeny as EM angiosperms showed slightly higher MBC in the O horizon compared to the A horizon (Table S4.4). In the A horizon, both EM angiosperms and gymnosperms had similar levels of MBC, but in the O horizon EM angiosperms had significantly higher amounts of MBC than EM gymnosperms ($t = 2.96$, $p = 0.015$, $df = 32$). Although microbial biomass C varied by mycorrhizal type and phylogeny, microbial C to N ratios were consistent across horizons, mycorrhizal type, and phylogeny (Table S4.3).

Bacteria made up the majority of the microbial community across mycorrhizal types and phylogeny. While there were significant differences in the fungal:bacterial ratios by mycorrhizal type and phylogeny, these were relatively minor as the fungal:bacterial ratio only varied from 0.08 – 0.15. There were slightly more fungi in both EM angiosperm and EM gymnosperm plots (Fig. S4.6), and EM angiosperms had significantly higher fungal:bacterial ratios compared with the both AM ($t = -3.36$, $p = 0.012$) and EM gymnosperm ($t = 2.79$, $p = 0.047$) plots. However, fungal abundance was always lower than bacterial abundance.

Edaphic and other factors

Most soil factors varied primarily by mycorrhizal type, though soil pH varied by both mycorrhizal type and phylogeny. EM gymnosperm plots showed the lowest soil pH values, followed by EM angiosperms and then AM angiosperms (Table 4.4, S4.3, S4.4). AM angiosperm plots averaged a full soil pH unit higher than EM gymnosperm plots. AM plots also had notably higher cation exchange capacity, percent base saturation, and *Lumbricus terrestris* biomass than EM plots (Table S4.3, S4.4 and Figure S4.7). While percent clay was also higher in AM angiosperm plots, overall clay content was still relatively low (mean AM = 7.7 +/- 3.1 %, EM = 2.7 +/- 1.8%). Additionally, for some variables, species accounted for a large portion of the variation explained by the model (i.e. *L. terrestris* biomass) while for other variables it accounted for almost no variation (i.e. CEC, % Clay) (Table S4.3).

Multi-variate assessment of the nutrient economies of AM angiosperms, EM angiosperms, and EM gymnosperm plots

In the PCA, the first principal component explained most of the separation between AM angiosperms, EM angiosperms, and EM gymnosperms. The first two principal components explained 43.8% of the variation in the data (PC1 = 24.5%, PC2 = 19.4%), and the variables contributing most to PC1 and PC2 are highlighted in Table 4.6. Notably, the major contributors to PC1 included forest floor turnover (14.8%), total C and N from the O horizon (13.5% each), and NO_3^- resin accumulation (9.4% A horizon, 6.3% O horizon). PC2 showed a small amount of vertical separation between EM

angiosperms and the other two groups, but EM gymnosperms and AM angiosperms had similar positions on that axis (Figure 4.6). The strongest contributors to PC2 were related to rates of N cycling, including potential N mineralization and potential nitrification (A horizon, 15.3% and 10.2%, respectively), net nitrification and net N mineralization (13.9% and 10.9% respectively), and DOC (O horizon, 9.7%). Higher PC1 scores were positively correlated with O horizon C and N, NO_3^- accumulation on resin in the A and O horizons, and slower forest floor turnover rates and lower DON:DIN ratios (Table S4.5). Higher PC2 scores were negatively associated with rates of N cycling and MBC, but positively associated with DOC in the O horizon.

Discussion

Understanding species effects on C and N cycling is a critical challenge for ecosystem ecologists. Here, we examined whether the patterns predicted by the MANE framework would be found in forests that started from the same initial soil conditions, and whether phylogeny could be used in addition to the MANE framework for predicting C and N dynamics among EM species, including both gymnosperms and angiosperms. We hypothesized that EM gymnosperms would exhibit a more organic nutrient economy than EM angiosperms due to more recalcitrant litter and lower soil pH slowing key C and N cycle processes. We tested our hypothesis using a common garden experimental design and compared key metrics of C and N cycling by mycorrhizal type, phylogeny, or both together. Overall, mycorrhizal type and phylogeny both influenced multiple metrics of C

and N cycling; however, mycorrhizal type and phylogeny were usually not included in the best-fit models together, suggesting that they are important for different processes.

Both mycorrhizal type and phylogeny influence C and N dynamics

We found broad support for key tenets of the MANE framework overall, as well as partial support for our hypothesis that EM gymnosperms would exhibit a more organic nutrient economy than EM angiosperms. The C and N cycling metrics that supported our first two hypotheses of clear separation of AM angiosperms, EM angiosperms, and EM gymnosperms are listed in the first column of Table 4.5 and are less numerous than the columns showing differences primarily by mycorrhizal type or phylogeny. These metrics also tended to be constrained to measurements from the upper soil horizons (O horizon or top 10 cm of mineral soil) and fell along the expected axis of inorganic-organic nutrient economies, with AM angiosperms having higher rates of C and N cycling and lower C storage than EM gymnosperms. However, most metrics only showed differences by mycorrhizal type (e.g. nitrogen mineralization, leaf litter *k*) or by phylogeny (e.g. total soil C and N storage, DON:DIN ratios). Our PCA helped to summarize these differences and suggests that the combination of the differences by MF type or by phylogeny resulted in the expected groupings of species along an organic-inorganic nutrient economy, as most separation of the three groups happened across one principal component. However, we have some indication that EM gymnosperms cannot simply be considered as more "extreme" than EM angiosperms along the inorganic-organic continuum of the MANE framework as they do not always have a more organic nutrient economy, as the PC2 axis

demonstrates. Additionally, the greatest differences between MF types and phylogenetic groups did not always follow the expected patterns; for example, the DON:DIN ratios were lowest for EM gymnosperms rather than highest. Therefore, in the following paragraphs we describe the key trends leading to the distinction of these groups and investigate why they did/did not match our expectations. As previous studies have delved into many of the mechanisms explaining differences in pools and processes (e.g. Hobbie *et al.* (2006, 2007b, 2010); Mueller *et al.* (2012a)), in the following paragraphs we focus mainly on how these patterns contributed to differences between the groups in their nutrient economies as expected or unexpected and the potential implications.

Differences in decomposition rates and biomass inputs contribute strongly to separation of AM angiosperms, EM angiosperms, and EM gymnosperms

As one of the primary drivers of the MANE framework, we expected differences in decomposition rates by mycorrhizal type and phylogeny that would lead to the expected differences in dominance of organic versus inorganic nutrient economies. Results from leaf and fine root decomposition experiments and measurements of biomass inputs supported our hypothesis that EM gymnosperms would have a more organic nutrient economy, as the three groups differed in decomposition rates and organic inputs to soils that have implications for total C and N storage in the upper horizons. EM gymnosperm litter had the lowest estimated leaf litter decomposition rates of the three groups, especially when considering forest floor turnover; both suggest that there would be more nutrients tied up in organic material in EM gymnosperm plots than in either AM

or EM angiosperm plots. This is indeed what we observed: even though AM angiosperm plots had substantially larger plant biomass inputs, the rapid turnover rates of their leaf and root litter likely prevents the buildup of a thick O horizon, evidenced by the limited C and N pools in the O horizon. Our results are supported by numerous studies of decomposition highlighting the more rapid decomposition of AM litter vs. EM litter in both roots and leaves (Jacobs *et al.*, 2018; Keller & Phillips, 2019; See *et al.*, 2019), and follows our prediction that EM gymnosperm leaf litter would decompose the slowest due their lower quality litter as EM gymnosperms had the highest Lignin:N ratios and C:N ratios. Litter quality differences were not associated with faster root decomposition in the same way, but see section "*Fine roots contributed …"* for additional discussion.

In addition to locking up nutrients in the organic horizon, the differences in decomposition rates by mycorrhizal type and phylogeny have implications for both total C storage and stabilization. While the C stored in the organic horizon may not turn over as rapidly in EM gymnosperm plots as AM angiosperm, it still has a much shorter mean residence time than soil C that is chemically stabilized onto mineral surfaces (Lutzow *et al.*, 2006; Kögel-Knabner *et al.*, 2008) or physically protected in soil aggregates (Jastrow *et al.*, 1996). Recent research has emphasized that soil C with long mean residence times is more likely to be the byproducts of microbial processing (Cotrufo *et al.*, 2013, 2015), which is promoted in systems with higher quality litter that can be more rapidly incorporated into microbial biomass (Hobbie *et al.*, 2007). The greater biomass of earthworms, especially *L. terrestris*, observed in the AM plots could be associated with increased microbial processing (Groffman *et al.*, 2015) as well as increases in the amount of C protected in soil aggregates (Fahey *et al.*, 2013), both of which promote the

formation of more stabile soil C in AM plots. Soil aggregation may also be augmented by the AM fungi themselves (Frey, 2019). Thus, the C stored in AM angiosperm soils could be more stable than the C in the EM plots as inputs decompose rapidly and are likely incorporated into microbial biomass given the larger amount of microbial biomass C in AM angiosperm plots compared to EM plots. While some recent studies have come out exploring differences in C stabilization in EM versus AM dominated systems (Craig *et al.*, 2019; Frey, 2019), it remains an area in need of additional research.

Most N cycling metrics explain intra-group variation rather than variation between groups

Given the large differences in inputs and decomposition rates, we expected to see substantial separation of AM angiosperms, EM angiosperms, and EM gymnosperms in metrics related to N cycling and organic nutrient pools. However, few metrics related to organic nutrient pools or N cycling played a role in separating the groups along the PC1 axis, and those that did sometimes differed in unexpected ways. For example, while DON:DIN contributed to separation of angiosperms and gymnosperms, the DON:DIN ratio was highest for angiosperms, not gymnosperms as we expected, and was at least in part driven by differences in the size of the DON pool not just the DIN pool (data not shown). The other N cycling metrics that contributed to PC1 were the accumulation of DIN on resins, which interestingly was much higher in EM gymnosperm plots compared to the angiosperm plots. We had expected greater N accumulation of DIN for AM

angiosperm plots since they tended to have slightly higher rates of *in situ* N mineralization and nitrification.

These unexpected differences in DON:DIN ratios and DIN accumulation on resins could be explained by a few factors. First, though we expected to see more DON production in EM gymnosperm plots, since our metric of DON came from laboratory soil incubations any EM fungi present would have lost their carbon source and would likely be unable to contribute to the breakdown of organic matter (Lindahl & Tunlid, 2015). Since EM fungi can produce extracellular enzymes to mine organic matter for acquiring nutrients, e.g. β-N-acetylglucosaminidase among others (Courty *et al.*, 2010; Pellitier & Zak, 2018), the lack of these organisms in incubations may mean that the DON measurements are less indicative of the *in situ* availability of DON. The greater DON concentrations in the AM angiosperm plots could also be due to a larger proportion of saprotrophic fungi in the incubations, as AM fungi may not compete as directly with saprotrophic fungi as EM fungi do (Gadgil & Gadgil, 1971; Averill *et al.*, 2014).

Second, since resin accumulation differs from our other metrics of N cycling in that it is influenced by plant N uptake as well as microbial uptake (and processing), we could be capturing differences in the types of N taken up by gymnosperms compared to angiosperms. Gymnosperms (and their associated mycorrhiza) have been documented to take up N in organic forms (Gallet-Budynek *et al.*, 2009; Boczulak *et al.*, 2014; Liu *et al.*, 2017), which might be expected here given the large accumulation of nutrients tied up in their O horizon, and could account for the larger DIN pools on the resin if EM gymnosperms are not taking up as much NH_4^+ or NO_3^- , allowing them to accumulate on

the resin. This mechanism is supported by our data on the potential rates of mineralization and nitrification, as differences between gymnosperms and angiosperms were small and highly variable, indicating that all groups have similar capacity for mineralization and nitrification.

Finally, if EM gymnosperms have lower overall N demand than the EM or AM angiosperms, that would result in larger DIN accumulation. Since these species tend to have longer leaf lifespans they may retain more N and have lower uptake requirements (Reich *et al.*, 2007). Additionally, since the EM gymnosperms had low fine root ingrowth and the lowest fine root biomass (data not shown), lower uptake could also be due to lower root length density (length of root per volume of soil) in these species. Root length density influences the total amount of soil explored for nutrients (Jackson *et al.*, 2008; Fort *et al.*, 2014), and thus the total amount of nutrients available for uptake. Less uptake would enable greater accumulation of N on resins that we observed for EM gymnosperms, but there has yet to be substantial documentation of differences in overall N uptake and the uptake of specific N forms by mycorrhizal type (Lin *et al.*, 2017).

Most of the other N cycling metrics and the organic C pool significantly contributed to PC2, suggesting that they help explain variation in the data even if this contributed little to the separation between groups. DOC pools showed relatively small differences by mycorrhizal type or phylogeny though they did contribute to separation of plots along the PC2 axis, but this is potentially related to variation among species within a group, as most of the variation explained by the DOC models came from the species random effect. In contrast to DOC, variation across species explained little variation in

net N mineralization and net nitrification though they had strong contributions to PC2, as for both metrics the models had extremely low explanatory power and the random effect did not help explain additional variation in the data. However, while PC2 does not contribute to separation of species by mycorrhizal type and phylogeny, there does appear to be a gradient of more organic to more inorganic C and N cycling with increasing values of PC2. For example, higher rates of N cycling (in situ and laboratory incubations) were positively correlated with this axis, while DOC and total pools of C and N in the O horizon were negatively correlated with PC2. The AM angiosperms also clustered with higher values of PC2, which again suggests that they have an inorganic nutrient economy, while there was substantial variation among EM species, and especially EM angiosperms, along this axis.

The similarity between groups in terms of N cycling and DOC did not match our prediction, but is not entirely unexpected. Measurements of *in situ* N cycling rates are not only influenced by the quality of the litter, but also the environment and microbial community (D'Amore *et al.*, 2009; Butler *et al.*, 2012; Waring *et al.*, 2016). Even though environmental conditions should vary less in a common garden than in natural systems, studies have shown that within-plot variation in N cycling is substantial and microclimate conditions could vary enough to influence these rates (Augusto *et al.*, 2015; Waring *et al.*, 2016) see section "*Soil properties and organisms…*" for more discussion). This could also be caused by the difference between plantations/common gardens and natural ecosystems. For example, N mineralization rates in stands of (EM) gymnosperms in plantations were higher than those in natural stands and comparable to rates found in natural angiosperm stands (Mueller et al. 2012). This could be related to differences in

the microbial communities between natural stands versus plantations, as our EM gymnosperm plots had lower EM species richness and fungal:bacterial ratios than EM angiosperms plots, which may not be the case in natural stands.

Whole soil C but not N pools contribute to separation of MF type and phylogeny

We had expected that soil N pools would be smaller under EM species, especially EM gymnosperms, given their lower quality litter and the ability of EM fungi to mine organic matter for N. However, similar to our finding that N cycling showed little separation between AM angiosperms, EM angiosperms, and EM gymnosperms, whole-soil N pools did not differ substantially between these groups even though both EM angiosperms and gymnosperms species had lower quality litter. Soil N pools also remained relatively constant with depth in the mineral soil. In contrast, soil C declined in each 10cm depth increment, except for AM and EM angiosperms between the O horizon and top 10cm of mineral soil (Fig. S4.1). Additionally, only whole-profile C content was correlated with PC1, while soil N was only correlated with PC1 for the O horizon. This differs from what other studies have found in comparing soil C and N under AM versus EM species, where soil N was lower for EM species and was responsible for greater C:N ratios found under EM vs. AM species (Lin *et al.*, 2017; Zhu *et al.*, 2018; Lyu *et al.*, 2019). This could in part be due to the fact that prior to planting, the entire common garden had been a plantation of EM gymnosperms (*Pinus sylvestris*). Therefore, nitrogen may have already been low in this system and the influence of AM angiosperms may not have been strong enough to increase soil N pools as observed in natural systems (Lin *et*

al., 2017). Additionally, the common garden could have less diverse or different compositions in the EM fungal community, which could mean that fewer species were present with significant decomposer capabilities which would impact the ability of the EM community to reduce soil N as observed in the other studies (Lin *et al.*, 2017). Finally, even though substantial time had passed since planting, the depletion of soil N observed by other studies may take even longer, and continued monitoring of soil C and N would be needed to determine if soil N declines over time.

Fine roots contributed little to our measured differences in C and N cycling by mycorrhizal type and phylogeny

In contrast to our third hypothesis, root inputs seemed to contribute little to the separation of nutrient economies between AM angiosperms, EM angiosperms, and EM gymnosperms. Neither fine root ingrowth nor fine root decomposition were strongly correlated with PC axes 1-3, though total inputs that included roots did contribute to PC1. We also had expected less separation in root traits and inputs by group since roots are used to acquire multiple resources and may not correlate as strongly with aboveground traits (Weemstra *et al.*, 2016). This was partially true within AM and EM angiosperms but not EM gymnosperms, as fine root decomposition, ingrowth, and chemical quality all differed by phylogeny. We hypothesized earlier that N uptake may differ between these stands either as a function of uptake rates (physiology), differential uptake of different N forms (Öhlund & Näsholm, 2001; Pritchard & Guy, 2005), or differences in whole plant N acquisition caused by differences in root length density (Jackson *et al.*, 2008; Fort *et*

al., 2014). Differences in N acquisition could be more directly tested, and different root traits or processes could be measured to determine if root differences really are less important or we simply need to measure different aspects of root influences on the nutrient economies in these plots.

Another explanation for the weak contribution of roots to separation of the three groups could be that roots influence aspects of the nutrient economy that we did not quantify. Belowground inputs through roots and mycorrhizae may be more important in forming stabile C pools (Godbold *et al.*, 2006; Clemmensen *et al.*, 2013) and in some cases have been shown to differ by mycorrhizal type (Chen *et al.*, 2016), yet we only quantified total C pools. As mentioned previously, the faster processing of AM litter could potentially favor the formation of stabile, mineral-associated organic matter in those plots (Cotrufo *et al.*, 2015), so the faster decomposition rates we observed for fine roots compared to leaf litter could also accentuate these differences by MF group and phylogeny.

Finally, the mycorrhizal fungi themselves, rather than tree roots, could be more important than fine roots for explaining differences by MF type and phylogeny in the nutrient economies. Numerous studies show the strong influence of tree species on litter and soil microbial communities (Urbanová *et al.*, 2015; Cheeke *et al.*, 2017; Rożek *et al.*, 2020) and demonstrate that the fungal community shifts from more saprotrophic to more mycorrhizal with increasing soil depth (Carteron *et al.*, 2020). While we examined differences in species richness of EM fungi and saprotrophic fungi, these data do not describe the functioning of the fungal community, which can have distinct impacts on

ecosystem processes (Wurzburger *et al.*, 2017; Delavaux *et al.*, 2017). For example, traits like hyphal turnover and ingrowth as well as enzyme production could help better quantify belowground differences in the nutrient economies, and could also help explain some of our unexpected patterns in N cycling (Godbold *et al.*, 2006; Jones *et al.*, 2010; Talbot *et al.*, 2013; Soudzilovskaia *et al.*, 2015; Wurzburger & Brookshire, 2017). Decomposition of fungal necromass could also be a substantial input to soil C, though the factors influencing decomposition of fungal necromass are still an active area of research (Fernandez & Koide, 2014; Fernandez *et al.*, 2016). Overall, the mechanisms by which roots and belowground inputs and microorganisms contribute to differences in the nutrient economies of these different groups still has great potential for future research.

Soil properties and organisms help explain variation in N cycling

We found multiple environmental or soil properties that helped explain more variation in N cycling than mycorrhizal type and phylogeny. Additionally, these properties tended to vary among the three groups, which helps explain the small observed differences in N cycling in our study. For example, average soil moisture was positively correlated with net N mineralization, and incorporating this relationship eliminated the small differences we saw by mycorrhizal type because AM angiosperm plots had higher soil moisture than EM angiosperm or gymnosperm plots. Differences in soil moisture could in part be caused by differences in phylogeny, as EM gymnosperm plots can intercept more precipitation and have drier soils than angiosperms (Augusto *et al.*, 2015). Drier soils could also influence the microbial community, as EM trees increased root

exudation under drought conditions compared to AM trees (Liese *et al.*, 2018). Similar to net N mineralization, incorporating covariates into the model of net nitrification (fungal:bacterial ratio) significantly increased the explanatory power of the model but did not distinguish nitrification rates by MF type of phylogeny. Rather, all groups showed a significant decline in nitrification rates with increasing fungal:bacterial ratios, and as EM angiosperms had the highest ratio of fungi:bacteria, they tended to have lower nitrification rates when considered separately. Finally, in contrast to N mineralization and nitrification, the DON:DIN ratio was positively correlated with EM species richness, which partially explains the lower DON:DIN ratio in EM gymnosperm plots as they had lower EM richness than EM angiosperm plots. However, even after accounting for that, EM gymnosperms trended lower in their DON:DIN ratio than EM angiosperms. This suggests that retaining the broader group classification (here phylogeny) can sometimes remain and be useful in distinguishing these processes even after incorporating additional information about the soil properties or community.

That we did not find clear differences in N cycling by mycorrhizal type and phylogeny after accounting for soil properties or the microbial community does not necessarily mean that these results contradict the MANE framework. Rather, we may be seeing how the common garden approach differs from measuring these processes in natural stands. Microbial communities may be more diverse in natural stands that have greater tree species diversity than the common garden (Burton *et al.*, 2010; Wang *et al.*, 2017), and we know that the microbial community plays an especially important role for these processes (Wurzburger *et al.*, 2017; Cheeke *et al.*, 2017). Additionally, the patterns we observed with our covariates for N cycling match predictions made by the MANE

framework. For example, DON:DIN ratios increased with EM species richness, similar to the MANE framework's DON:DIN ratios increasing with greater EM basal area. Similarly, the MANE framework proposed that EM stands will have greater fungal:bacterial ratios, and we found that nitrification declined with higher fungal:bacterial ratios. In essence, the monoculture plots could be fostering microbial communities atypical of the natural stands under which the framework was developed, which may have reduced the signal in our data relative to the variation common in measurements of N cycling. Conducting this analysis in natural stands or experiment plots with multiple gymnosperm species would help determine whether N cycling may contribute more to separation by MF type and phylogeny along the PC1 axis, or if there remain differences between gymnosperms and angiosperms that warrant a separate axis as in our study.

Implications and Conclusions

Through this work we have shown support for a multi-trait framework using both MANE and phylogeny as foundations, highlighting that AM and EM angiosperms and EM gymnosperms each warrant their own classification. The use of a common garden for this analysis highlights the role of the species in creating the distinct nutrient economies, rather than AM species simply being more likely to occur on soils with more inorganic nutrient availability than EM species, and vice versa. Our analysis also showed that for the most part, the separation of EM gymnosperms into a more organic nutrient economy occurred due to a combination of differences between EM and AM species and between

angiosperms and gymnosperms, rather than differences among all three groups. For example, total organic inputs to soils contributed to separation of the three groups, but was due to differences in aboveground inputs by phylogeny and belowground inputs by MF type. Additionally, soil C shifted from differing primarily by MF type in the upper horizons to primarily by phylogeny when considering the whole profile. Since differences resulted from this combination, it would be interesting to determine whether AM gymnosperms, which remain relatively unstudied in this framework as well, could behave similarly to EM gymnosperms and angiosperms in some cases and AM angiosperms in others. Applying this framework accurately to gymnosperms will therefore require additional research to determine which processes are controlled more by phylogeny than mycorrhizal type.

It is also important to consider how the results of this study could differ if the common garden had been established in a different location. If the common garden was in a cooler climate, most C and N cycling processes would likely happen more slowly. A reduction in the rates of C and N could enhance differences between groups if rates declined more for EM angiosperms or gymnosperms than AM angiosperms. It would also be illuminating to conduct a similar experiment on a previously AM-forested site, as this could have influenced our results and would help determine the role of species and their associated microbial communities on establishing these nutrient economies. Research on the role of fungi versus plants in establishing these patterns is also necessary as we found evidence that within EM species, differences in the microbial community could account for why gymnosperms did not have a more organic nutrient economy than EM angiosperms. Additional comparisons of C and N cycling in AM gymnosperms could

help elucidate how plant traits versus fungi contribute to the distinct nutrient economies observed in stands dominated by different species groups.

Overall, the distinct nutrient economy of EM gymnosperms highlighted here has important implications for application of the MANE framework, as it shows that treating EM species as one category would miss a substantial amount of variation that occurs in C and N cycling between angiosperms and gymnosperms. Combining phylogenetic and the MANE frameworks will allow them to be used more broadly in global C models, as ecosystems like the boreal forest are dominated by one mycorrhizal type but have both gymnosperms and angiosperms.

Table 4.1. List of study species and their common names, mycorrhizal type, phylogeny, and number of replicate plots. Species with 6 replicates were planted in both blocks.

Species name	Authority	Common name	MF Type	Phy	Plots
Abies alba	Mill.	Silver fir	EM	Gym	2
Acer platanoides	L.	Norway maple	AM	Ang	3
Acer pseudoplatanus	L.	Sycamore	AM	Ang	3
Betula pendula	Roth.	Silver birch	EM	Ang	3
Carpinus betulus	L.	European hornbeam	EM	Ang	3
Fagus sylvatica	L.	European beech	EM	Ang	3
Larix decidua	Mill.	European larch	EM	Gym	6
Picea abies	(L.) Karst	Norway spruce	EM	Gym	6
Pinus nigra	Arn.	Austrian black pine	EM	Gym	3
Pinus sylvestris	L.	Scots pine	EM	Gym	3
*Pseudotsuga menziesii**	Franco	Douglas fir	EM	Gym	6
Quercus robur	L.	English oak	EM	Ang	6
*Quercus rubra**	L.	Red oak	EM	Ang	3
Tilia cordata	Mill.	Small-leafed lime	EM	Ang	3

*denotes species of North American origin; MF type = mycorrhizal type; Phy = phylogeny

Table 4.2. Summary of model fits for dependent variables of interest. In cases where the LM fit the data better but variance for random effects could still be estimated, we present the results for the LMM. In cases where multiple models had AIC and BIC values within 2, we present the model with the lowest AIC value first followed by the next model(s) in parentheses.

Property/Dependent Variable	Best Model Type	Best Model(s)	R^2*	ICC	RMSE
Litter Decomposition (Home Plot)	LMM	(MF)	0.30, 0.07	0.24	0.1
Forest Floor Turnover	LMM	MF + Phy	0.62, 0.46	0.28	0.28
Root decomposition (Common Plot)	LMM	Phy	0.10, 0.09	0.02	0.08
Leaf litterfall	LMM	Phy	0.71, 0.23	0.63	0.11
Root Ingrowth	LMM	MF	0.37, 0.22	0.2	0.18
Total Inputs (leaf + root)	LMM	Phy (MF + Phy)	0.68, 0.25	0.58	0.09
Net N mineralization	LM (LMM)**	MF	0.08		0.21
Net Nitrification	LM**	MF	0.05		0.62
DON:DIN	LM (LMM)	Phy	0.36, 0.32	0.03	0.25
Total C (O + 75cm)	LM (LMM)	Phy (MF + Phy)	0.38, 0.19	0.24	0.09
Total C (O horizon)	LMM	MF + Phy (MF)	0.66, 0.37	0.46	0.23
Proportion C in top 10cm	LMM (LM)	MF (Phy)	0.38, 0.07	0.34	0.1
DOC (A horizon)	LMM (LM)	Phy (MF)	0.27, 0.09	0.2	0.18
DOC (O horizon)	LMM (LM)	MF (Phy)	0.20, 0.02	0.19	0.17
Total N (O + 75cm)	LM**	Phy (MF + Phy, MF)	-0.04		0.13
Total N (O horizon)	LMM	MF + Phy (MF)	0.63, 0.36	0.43	0.25
C:N (O horizon)	LMM	MF (Phy)	0.41, 0.02	0.4	0.05
C:N (Top 10cm)	LMM (LM)	MF (MF + Phy)	0.49, 0.28	0.29	2.54
C:N (O thru 75 cm)	LM**	Phy	0.15		0.13

*for LMMs, the first value is R^2 conditional (variation explained by fixed and random effects) and the second is R^2 marginal (variation explained by fixed effects only); for LM, the adjusted R^2 is presented; **indicates LM used because of singular fit w/random effect
Abbreviations: LMM = Linear Mixed-Effects Model; LM = Linear Model; ICC = Intraclass correlation coefficient (higher values = greater proportion of variance explained by the grouping variable)

Table 4.3. Pairwise comparisons testing for differences between species groups. Each test was conducted using the estimated marginal means from the model, and the significance test reports the pairwise comparison of those estimates. We only compared groupings that remained in the best fit model.

Property	Comparison	Significance test
Litter Decomposition (Home Plot, yr^{-1})	AM – EM	t = 1.62, p = 0.129, df = 14
Forest Floor Turnover (yr^{-1})	AM ang – EM ang	t = 2.94, p = 0.030, df = 12.4
	AM ang – EM gym	t = 4.93, p = 0.0009, df = 12.0
	EM ang – EM gym	t = 2.85, p = 0.040, df = 10.5
Root Decomposition (Common Plot, yr^{-1})	Ang – Gym	t = 1.86, p = 0.09, df = 7.97
Root Ingrowth ($g\ m^{-2}$)	Ang – Gym	t = 3.17, p = 0.007, df = 14.4
Litterfall ($g\ m^{-2}$)	AM – EM	t = 2.35, p =0.037, df = 11.9
Total Inputs ($g\ m^{-2}$)	Ang – Gym	t = 2.56, p = 0.025, df = 11.8
Net N mineralization ($g\ N\ m^{-2}\ yr^{-1}$)	AM – EM	t = 2.3, p = 0.026, df = 51
Net nitrification ($g\ N\ m^{-2}\ yr^{-1}$)	AM – EM	t = 1.892, p = 0.064, df = 51
DON:DIN	Ang - Gym	t = 4.73, p = 0.001, df = 8.92
Soil C (O horizon + 75 cm, $kg\ C\ m^{-2}$)	Ang – Gym	t = -2.64, p = 0.023, df = 11.3
O horizon C ($kg\ C\ m^{-2}$)	AM ang – EM ang	t = -2.46, p = 0.073, df = 11.8
	AM ang – EM gym	t = -3.73, p = 0.008, df = 11.5
	EM ang – EM gym	t = -1.81, p = 0.21, df = 10.8
Proportion C in top 10 cm	AM – EM	t = -1.46, p = 0.168, df = 13.4
DOC (A horizon) ($mg\ g^{-1}$ soil)	Ang – Gym	t = -1.783, p = 0.10, df = 11.1
DOC (O horizon) ($mg\ g^{-1}$ soil)	AM – EM	t = -0.807, p = 0.43, df = 14.5
Soil N (O horizon + 75 cm) ($kg\ N\ m^{-2}$)	Ang - Gym	t = 0.237, p = 0.81, df = 51
O horizon N ($kg\ N\ m^{-2}$)	AM ang – EM ang	t = -2.58, p = 0.059, df = 11.8
	AM ang – EM gym	t = -3.76, p = 0.008, df = 11.6
	EM ang – EM gym	t = -1.68, p = 0.26, df = 10.8
C:N (top 10 cm)	AM – EM	t = - 3.13, p = 0.008, df = 13.5
C:N (O horizon + 75cm min)	Ang – Gym	t = - 3.16, p = 0.0027, df = 51

Table 4.4. Summary table showing the mean (SD) for soil properties, litter chemistry, and litter inputs by mycorrhizal type and phylogeny. Model fits are presented in Table 4.2 and Table S4.3.

Property	AM ang	EM ang	EM gym	Best Model
pH (sat paste)	5.13 (1.04)^{A}	4.46 (0.29)^{B}	4.09 (0.36)^{C}	MF + Phy
Clay (%)	7.73 (3.1)^{A}	2.71 (1.78)^{B}	2.73 (1.75)^{B}	MF
CEC (mmolc per kg)	26.1 (10.78)^{A}	12.5 (4.99)^{B}	14.0 (5.55)^{B}	MF
Base Saturation (%)	45.8 (23.69)^{A}	20.0 (14.9)^{B}	16.5 (9.53)^{B}	MF
C:N (O horizon)	31.5 (6.01)	28.3 (3.37)	29.9 (5.96)	--
C:N (O horizon + 10cm)	9.95 (2.92) ^{A}	14.9 (3.44)^{B}	16.6 (3.07)^{B}	MF + Phy
Lignin:N (litter)	18.8 (2.75)	24.9 (8.41)	33 (11.7)	--
Lignin:N (root)	53.3 (7.97)^{A}	56.3 (18.5)^{A}	40.8 (9.45)^{B}	Phy
C:N (leaves)	59 (13.8)^{A}	45.5 (12.5)^{A}	67 (23)^{B}	Phy
C:N (roots)	66.4 (13.2)^{A}	71.8 (18.4)^{A}	56.2 (11.7)^{B}	Phy
Lf P (ppm)	1227 (275)^{A}	1588 (221)^{A}	1061 (380)^{B}	Phy
Rt P (ppm)	1129 (73.9)	1196 (273)	1131 (264)	--
Leaf litterfall (g m^{-2})	338 (140)^{A}	311 (164)^{A}	186 (86.7)^{B}	Phy
Root Ingrowth (g m^{-2})	202 (119)^{A}	84.3 (44.8)^{B}	85.1 (40.5)^{B}	MF
Total Inputs (g m^{-2})	540 (236)^{A}	395 (157)^{A}	271 (85.3)^{B}	Phy (MF+Phy)

Table 4.5. List of C and N cycling metrics organized by the influence of mycorrhizal type and phylogeny. Metrics were placed into columns based on the best fit determined by AIC + BIC values.

Grouping	MF + Phy	MF	Phy	None
Carbon Pools	O horizon C MBC (O, A)	%C in top 10 cm DOC (O) Root Ingrowth	Total Inputs Leaf Litterfall Total Soil C	DOC (A) Mineral soil C
Nitrogen Pools	O horizon N		DON:DIN Total Soil N Resin NH4, NO3 Soil N (O + 10 cm min)	
Carbon Fluxes	Forest Floor Turnover	Leaf Litter k (Home Plot)	Fine root k (Common Plot)	
Nitrogen fluxes		Net N min. Net nit.	Pot. Nit. rate	Pot. N min. rate
Soil properties and microbial community	Soil pH Fungal:Bacterial C:N (10 cm, min)	C:N (O) % Clay CEC % Base Sat. Earthworms	MBC to MBN	
Plant Traits			Leaf Lignin:N Leaf C:N Leaf P Root Lignin:N Root C:N Root P	

Table 4.6. PCA loadings for each input variable for the first 3 principle components. PCs 1-3 explain 51.8% of the variation in the data. Highlighted variables contribute more to the principle component than would be expected from uniform contributions of all variables. Grayed out text indicates variables that were not important contributors to PC1, PC2, or PC3.

Variable*	Loadings PC1	Loadings PC2	Loadings PC3
Net nitrification rate	0.000	**12.912**	2.278
Soil N (Whole Profile)	0.033	0.838	**8.849**
DOC (O horizon)	0.038	**9.174**	2.325
Net N mineralization rate	0.115	**10.639**	2.024
Potential N mineralization (A horizon)	0.201	**14.729**	2.313
C:N (O horizon)	0.313	0.004	**9.417**
MBC (A horizon)	0.593	**6.918**	0.958
Potential Nitrification (A horizon)	1.228	**8.423**	0.543
Fine root ingrowth	1.272	3.076	1.865
DOC (A horizon)	1.361	0.079	0.535
Potential Nitrification (O horizon)	2.045	**3.745**	0.003
Leaf litter *k* (Home Plot)	2.113	2.000	0.252
Potential N mineralization (O horizon)	2.645	0.693	**7.056**
C:N (Whole Profile)	2.956	1.505	**12.263**
Root decomposition (Common Litter)	3.192	0.053	0.970
MBC (O horizon)	3.199	1.484	**7.128**
Leaf Litterfall	**4.643**	0.694	**9.764**
Soil C (Whole Profile)	**4.997**	0.252	1.112
Total Inputs (Leaf + root)	**5.518**	0.141	**11.924**
DON:DIN (A horizon)	**5.781**	0.000	**5.533**
NO3 Resin (O horizon)	**6.513**	2.245	**3.799**
Soil C (O horizon)	**7.461**	**7.000**	0.002
Soil N (O horizon)	**7.509**	**6.558**	0.267
NH4 Resin (A horizon)	**8.244**	0.299	3.053
NO3 Resin (A horizon)	**8.964**	0.915	1.653
Forest Floor Turnover	**9.186**	**4.215**	1.952
NH4 Resin (O horizon)	**9.879**	1.410	2.163

*all variables log10 transformed before PCA

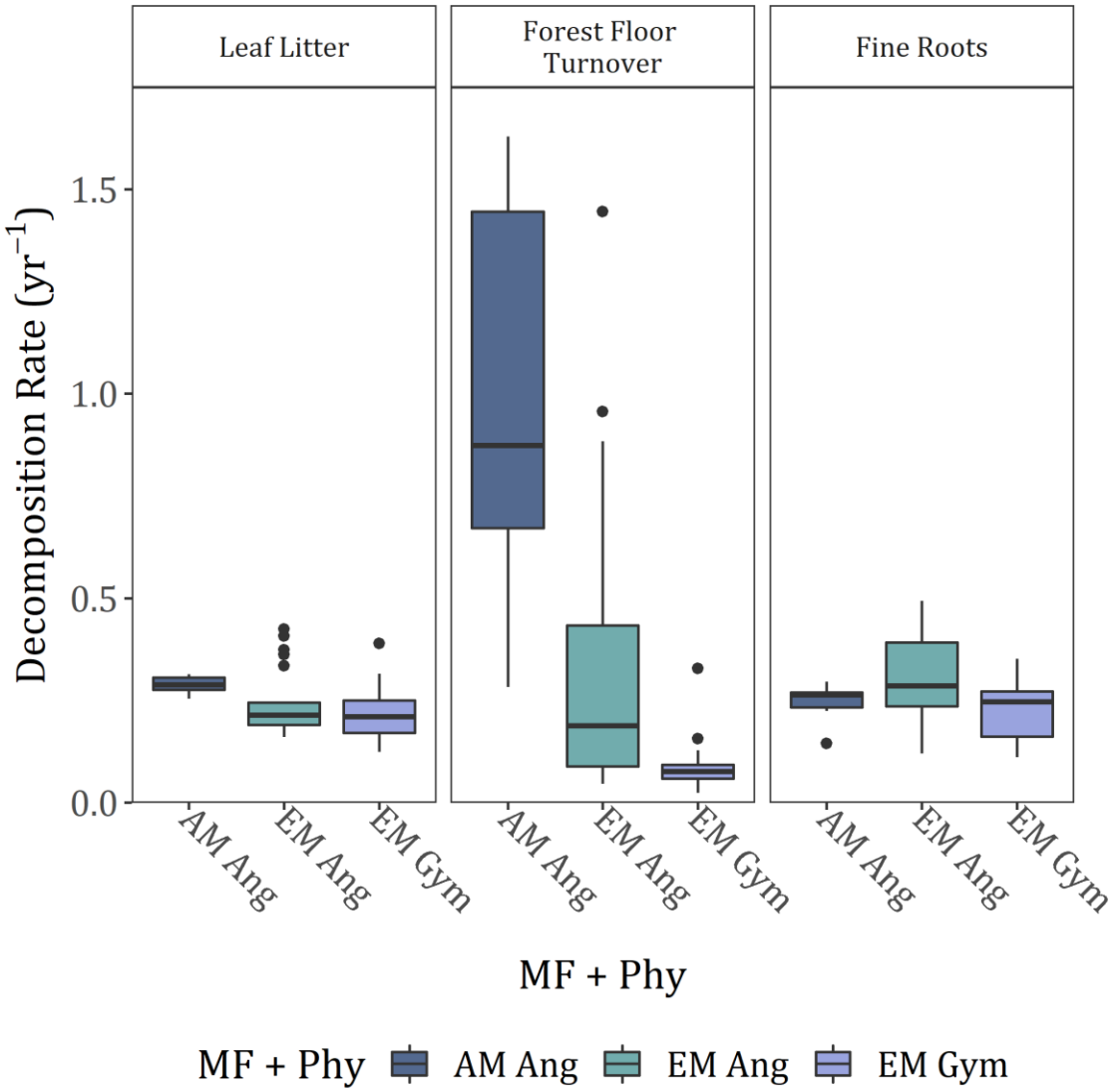

Figure 4.1. Influence of mycorrhizal type and phylogeny on leaf litter decomposition, forest floor turnover, and fine root decomposition (mean ± SE). Leaf litter and forest floor turnover were measured for each species in its home plot; fine root decomposition compares fine roots decomposing in a common plot. Leaf litter decomposition rates showed slightly higher decomposition rates for AM angiosperm litter compared to EM angiosperm and gymnosperm litter, but the difference was rather small ($t = 1.62$, $p = 0.13$, $df = 14$). However, forest floor turnover varied by both mycorrhizal type and phylogeny, with AM species having the fastest turnover and EM gymnosperms with the lowest turnover rate (4X lower than EM angiosperms). Fine root differed primarily by phylogeny, with roots decomposing faster for AM and EM angiosperms than EM gymnosperms ($t = 1.86$, $p = 0.09$).

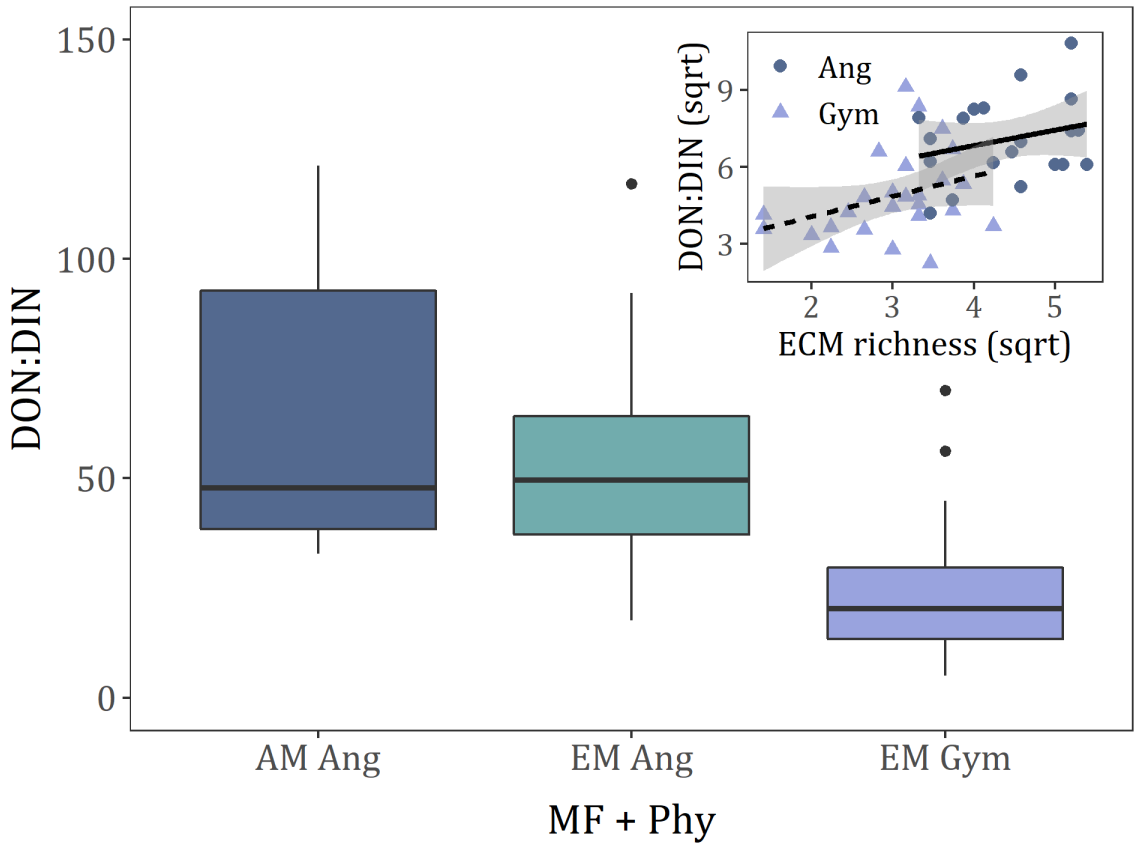

Figure 4.2. Ratio of dissolved organic N to dissolved inorganic N by mycorrhizal type and phylogeny, and according to EM richness in a plot (inset). EM gymnosperms tended to have lower DON:DIN ratios than either AM or EM angiosperms ($t = 4.73$, $p = 0.001$). In the inset, triangles represent EM gymnosperms and circles represent EM angiosperms, which excludes AM species. For EM plots, as the EM species richness increases, so does the ratio of DON:DIN ($R^2 = 0.43$). Additionally, EM gymnosperm plots had slightly lower DON:DIN ratios than EM angiosperm plots even after accounting for differences in EM richness ($t = 1.70$, $p = 0.10$).

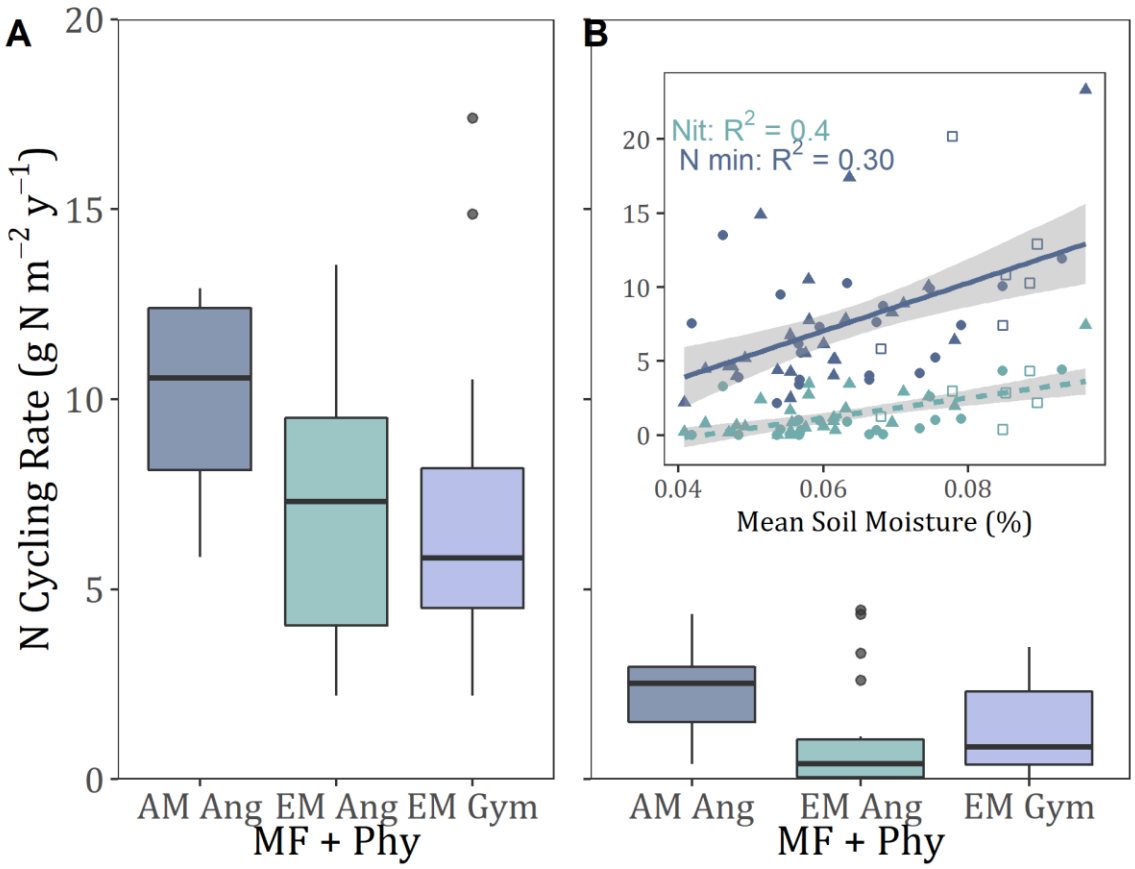

Figure 4.3. Net N mineralization and net nitrification rates vary by mycorrhizal type. Plot A shows rates of net N mineralization and plot B shows rates of net nitrification. The inset shows both N mineralization and nitrification rates versus percent soil moisture. In the inset, triangles represent EM gymnosperms, circles represent EM angiosperms, and open squares represent AM angiosperms. AM angiosperm plots had higher net N mineralization rates than EM angiosperm and gymnosperms ($t = 2.3$, $p = 0.026$). Net nitrification rates were only marginally higher in AM angiosperm compared to EM angiosperm and gymnosperm plots ($t = 1.89$, $p = 0.06$).

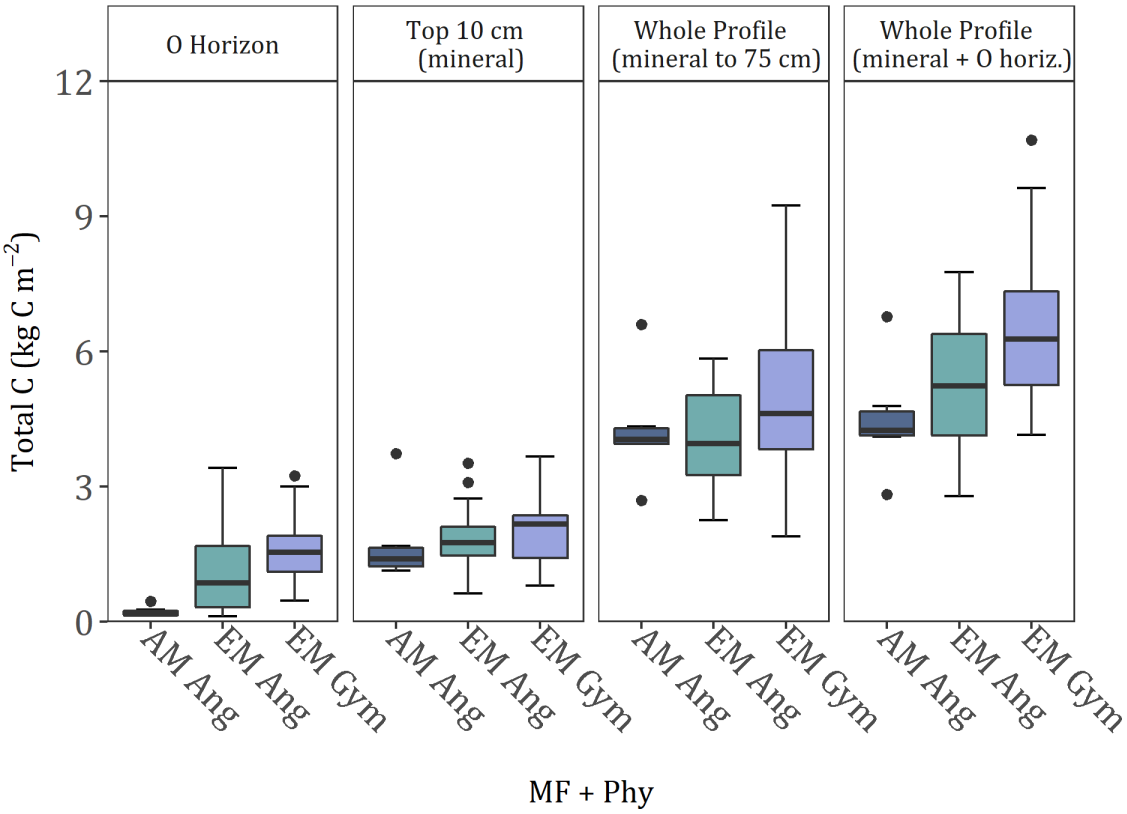

Figure 4.4. Total soil carbon for different soil depths down to 75 cm by mycorrhizal type and phylogeny. Total C down to 75 cm (including O horizon) differed primarily by phylogeny, and EM gymnosperms had the highest total soil C. However, O horizon carbon was greater for both EM angiosperm and EM gymnosperm plots compared to AM plots, though AM angiosperms had greater carbon in the lower horizons and so had similar total C to EM angiosperms.

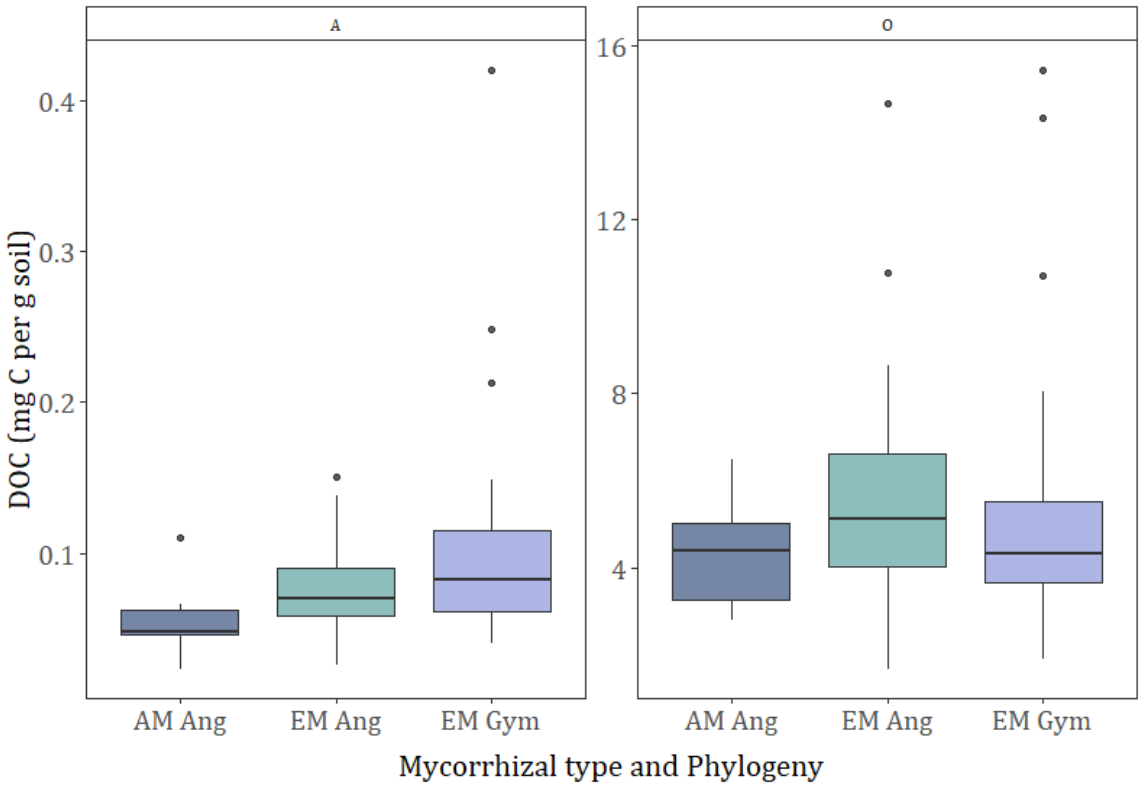

Figure 4.5. Dissolved organic carbon (DOC) by mycorrhizal type and phylogeny for the O and A horizons. Note the different scales for each horizon, as DOC levels were much higher in the O horizon compared to the A horizon.

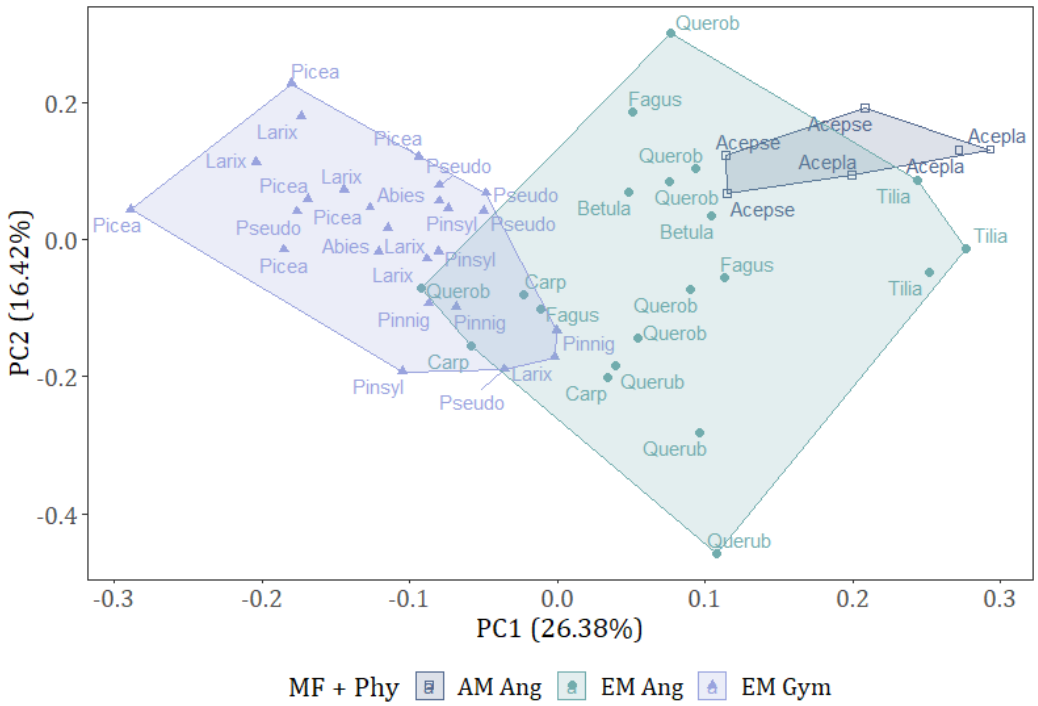

Figure 4.6. Principal component analysis showing clusters by mycorrhizal type and phylogeny. Points represent individual plots and are labeled with the abbreviated species name. Shapes represent the different groups as follows: Triangles = EM gymnosperms; Circles = EM angiosperms; Squares = AM angiosperms. Most of the distinction between the three groups was due to the PC1, with a small amount of separation on PC2. Additionally, most of the overlap between EM angiosperms and AM angiosperms is due to the three *Tilia cordata* plots, which haver values of PC1 than the other EM angiosperms.

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APPENDIX 1 – CHAPTER 1 SUPPLEMENTAL MATERIAL

Table S1.1. Mean trait values for three leaf traits related to the leaf economic spectrum for the study species. Trait values come from the GLOPnet database (Wright et al. 2004). Species are ordered from most to least acquisitive traits, with *B. papyrifera* having the most acquisitive traits of the study species and *P. glauca* having the least acquisitive traits. Trait values are presented as the mean ($\log_{10}$) along with the standard deviation; NA values indicate that only one observation for that species-trait combination was reported.

	Log Leaf Lifespan (months)	**Log Leaf Mass per Area (g m^{-2})**	**Log N_{mass} (%)**
Betula papyrifera	0.560 (NA)	1.88 (0.11)	0.360 (0.062)
Acer platanoides	0.78 (NA)	1.70 (0.05)	0.29 (0.11)
Acer saccharum	0.740 (NA)	1.83 (0.13)	0.26 (0.06)
Quercus rubra	0.78 (NA)	1.911 (0.12)	0.311 (0.064)
Larix laricina	0.78 (NA)	2.08 (NA)	0.13 (0.034)
Pinus strobus	1.30 (0.035)	2.09 (0.064)	0.149 (0.074)
Picea glauca	1.68 (0.17)	2.49 (0.04)	0.107 (0.037)

Table S1.2. Correlation coefficients between morphological traits across all species. All traits were ln-transformed before analysis.

	RTD	DIAM	RDMC
SRL	**-0.83*** **	**-0.66*** **	-0.04
RTD		**0.14** **	**0.17** **
DIAM			**-0.17** **

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table S1.3. Species differences in root morphological trait. Differences in traits by species were tested using GLS with variance weighted by species. Species differences were important for all traits measured, though at varying levels of significance.

Trait	Predictor	χ^2	p-value	Df
Specific Root Length	Intercept	5714.4	<0.0001	1
	Species	558.2	< 0.0001	6
	Block	0.12	0.94	2
Root Tissue Density	Intercept	12216.5	<0.0001	1
	Species	442.4	<0.0001	6
	Block	3.2	0.2	2
Diameter	Intercept	1405.6	<0.0001	1
	Species	178.2	<0.0001	6
	Block	16.3	0.00028	2
Root Dry Matter Content	Intercept	11633.6	<0.0001	1
	Species	18.0	0.0062	6
	Block	0.14	0.93	2

Table S1.4. Variance and weights for each GLS model examining trait differences by species. This highlights which species have more or less variance in each trait than the reference species, *P. strobus.* The variance for *P. strobus* is the same as the estimate for sigma (σ). Estimates greater than 1 indicate that species had more variance than *P. strobus* in that trait and estimates less than 1 indicate a species with lower variance in that trait.

	SRL		**RTD**		**Diameter**		**RDMC**	
Species	**estimate**	**95% CI**	**estimate**	**95% CI**	**estimate**	**95% CI**	**estimate**	**95% CI**
P. strobus	1.000		1.000		1.000		1.000	
B. papyrifera	0.792	0.602 - 1.041	1.266	0.952 - 1.682	0.951	0.732 - 1.234	1.323	1.012 - 1.729
P. glauca	0.739	0.559 - 0.976	1.298	0.975 - 1.729	0.613	0.466 - 0.806	0.996	0.758 - 1.308
L. laricina	0.711	0.542 - 0.932	0.960	0.723 - 1.273	0.843	0.645 - 1.103	0.784	0.600 - 1.024
Q. rubra	1.014	0.779 - 1.320	2.437	1.839 - 3.229	0.862	0.652 - 1.140	1.176	0.900 - 1.537
A. saccharum	0.479	0.364 - 0.630	0.978	0.734 - 1.302	0.569	0.428 - 0.756	0.875	0.671 - 1.143
A. platanoides	0.334	0.254 - 0.440	0.741	0.550 - 0.999	0.379	0.289 - 0.496	0.750	0.575 - 0.978
σ	0.807	0.666 - 0.978	0.384	0.313 - 0.470	0.266	0.220 - 0.323	0.223	0.185 - 0.269

Table S1.5. ANOVA table for the best fit GLS model of N uptake with each morphological trait, species, and N form. Uptake rates and root traits were log-transformed prior to analysis but are written without the log indicator for clarity.

Trait	Predictors	df	χ^2	p-value
Root Tissue Density	Intercept	1	1303.3	0.0000
	Species	6	24.5	0.0004
	N form	2	81.5	0.0000
	Root Tissue Density	1	41.8	0.0000
	Block	2	3.7	0.1550
	Species * N form	12	24.9	0.0150
	Species * Root Tissue Density	6	28.6	0.0000
Root Dry Matter Content	Intercept	1	1982.4	0.0000
	Species	6	26.3	0.0002
	N form	2	76.6	0.0000
	Root Dry Matter Content	1	14.8	0.0001
	Block	2	4.3	0.1150
	Species * N form	12	31.0	0.0019
Diameter	Intercept	1	6649.8	0.0000
	Species	6	15.1	0.0190
	N form	2	76.6	0.0000
	Diameter	1	6.8	0.0090
	Block	2	0.9	0.6400
	Species * N form	12	28.0	0.0060
	Species * Diameter	6	12.8	0.047

Table S1.6. Estimated slopes from GLS models of N uptake for each remaining morphological trait. Across-species average slopes and 95% confidence intervals are shown as well as the species-specific slope for the relationship between each trait and N uptake rate. RDMC did not interact with species so there is only one slope reported. All slope estimates are reported on the log-transformed scale.

	RTD		**DIAM**		**RMDC**	
Species	**Slope**	**95% CI**	**Slope**	**95% CI**	**Slope**	**95% CI**
Average	-0.116	-0.151 – -0.081	-0.136	-0.238 – -0.033	-0.128	-0.193 – -0.062
P. strobus	-0.224	-0.308 – -0.140	-0.317	-0.442 – -0.192		
B. papyrifera	-0.163	-0.228 – -0.098	-0.021	-0.166 – 0.125		
P. glauca	-0.147	-0.207 – -0.088	-0.226	-0.447 – -0.005		
L. laricina	-0.042	-0.127 – 0.044	-0.126	-0.249 – -0.003		
Q. rubra	-0.052	-0.110 – 0.006	-0.004	-0.240 – 0.232		
A. saccharum	0.024	-0.069 – 0.116	-0.181	-0.519 – 0.157		
A. platanoides	-0.208	-0.382 – -0.033	-0.074	-0.588 – 0.439		

RTD = root tissue density; RDMC = root dry matter content; DIAM = diameter

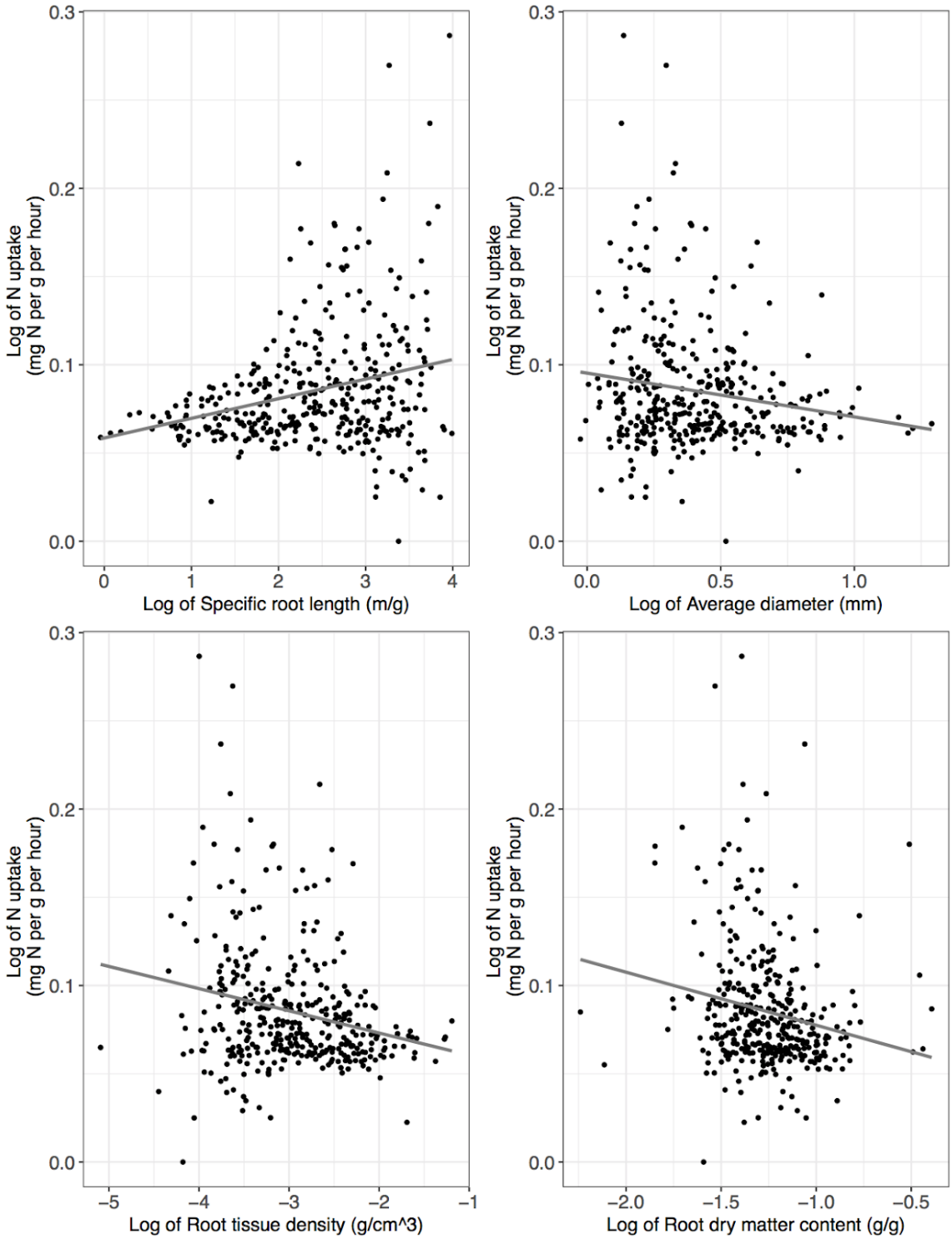

Figure S1.1. Morphological traits are related to N uptake rates. The figure shows log transformed N uptake rates, calculated from N depleted per gram root per hour, against log transformed morphological trait values. The unequal variance was accounted for by using generalized least squares method – the *gls* function from the *nlme* package.

APPENDIX 2 – CHAPTER 2 SUPPLEMENTAL MATERIAL

Table S2.1. Comparing binary logistic regression models of survival in *B. papyrifera*, *P. mariana*, and *P. resinosa*. N form did not improve the model fit according to the AIC and BIC values, though it did explain more variation than the model with just N level and species.

Predictors	AIC	BIC	R2_tjur
N Level * N Form + Species	113.32	137.51	0.21
N Level + Species	107.48	117.16	0.14

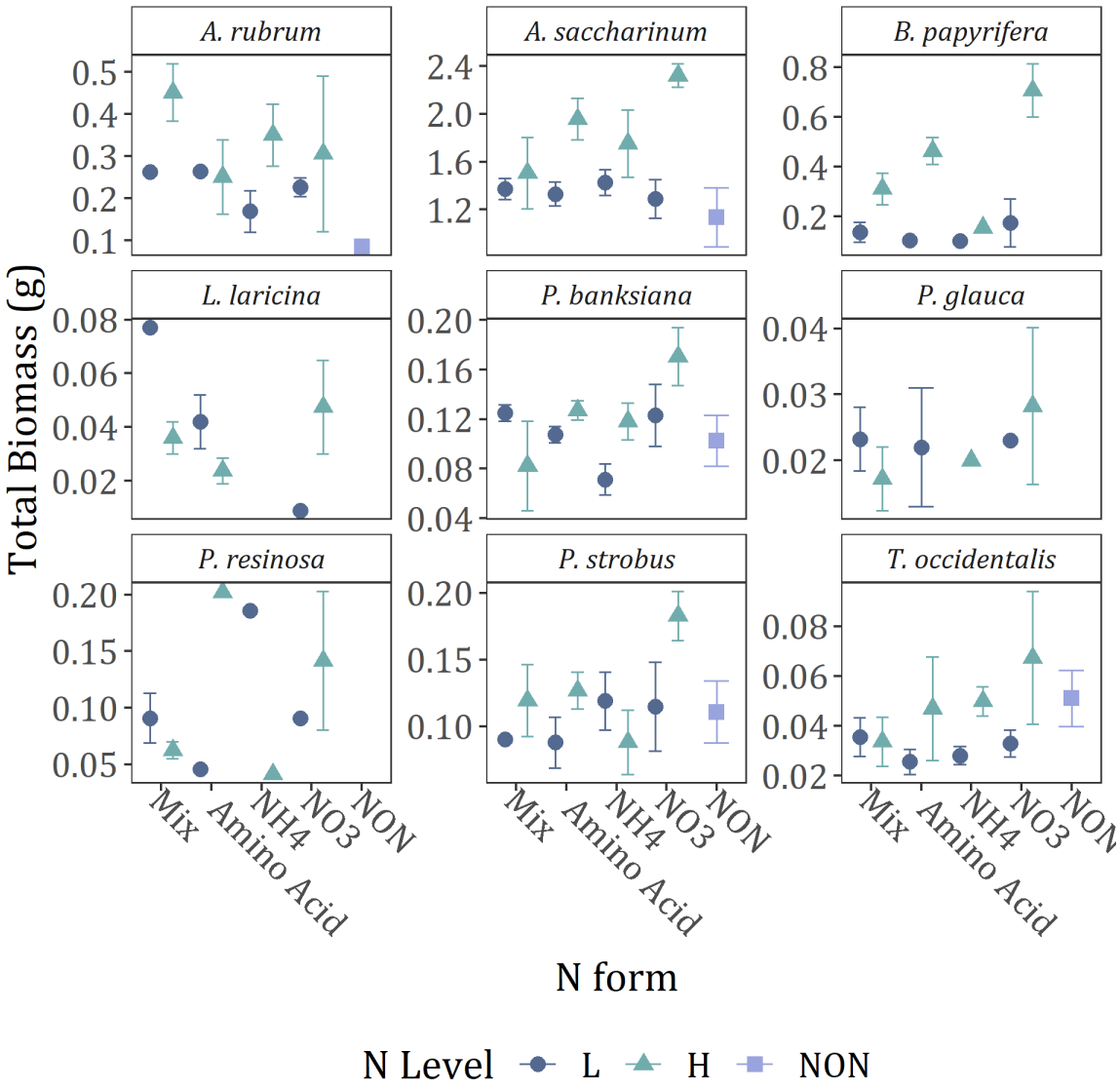

Figure S2.1. Mean biomass by species and treatment. Note the scale differs by species, and two species with very low survival (*A. balsamea* and *P. mariana*) are excluded from the figure due to low survival across treatments.

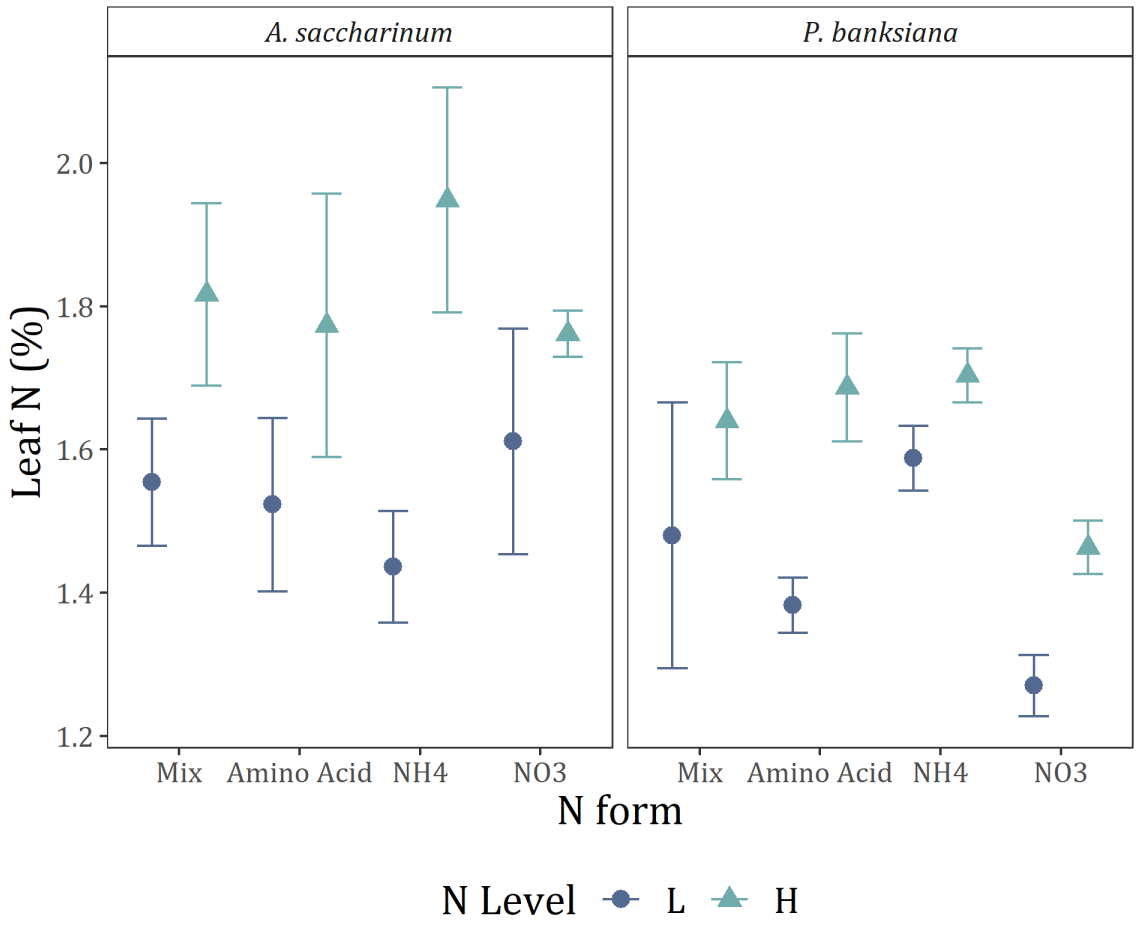

Figure S2.2. Leaf % N by species, N form and N level. N content was higher in leaves of *A. saccharinum* than *P. banksiana*, and at high versus low N levels, but did not vary significantly by N form.

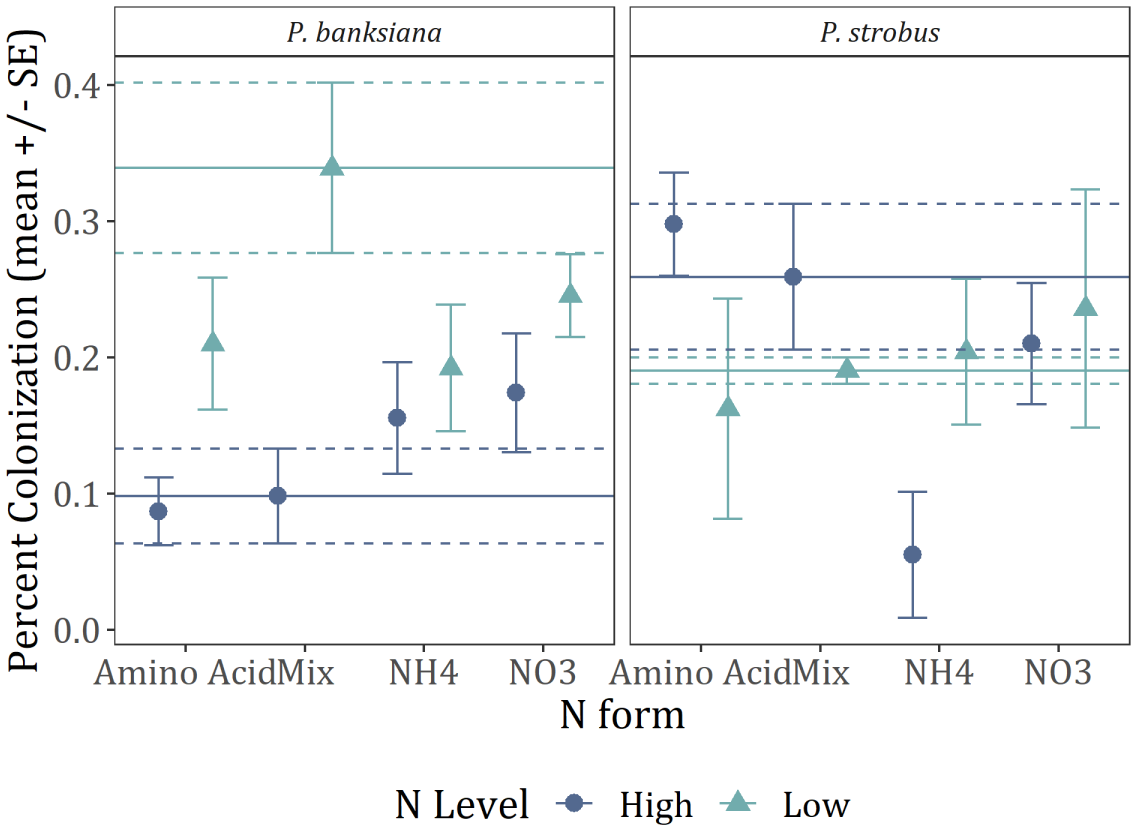

Figure S2.3. Colonization of root tips by ectomycorrhizal fungi on *Pinus banksiana* and *Pinus strobus*. The solid and dashed lines represent the mean % colonization +/- SE in the mixture treatment at each N level. The solid line that intersects the circle Mix treatment indicates the High N Level, while the solid line that intersects the triangle Mix treatment indicates the Low N Level.

APPENDIX 3 – CHAPTER 3 SUPPLEMENTAL MATERIALS

Table S3.1. Percent colonization of root tips by ECM fungi for *Quercus rubra* and *Picea glauca* by treatment and site.

			Mean % Colonization (SE)	
Heat	**Water**	**Site**	***Quercus rubra***	***Picea glauca***
Amb	Amb	CFC	54.8 (3.1)	59.3 (3.0)
Amb	Amb	HWRC	76.8 (2.8)	57.8 (2.2)
Amb	Dry	CFC	54.8 (8.1)	64.0 (2.9)
Amb	Dry	HWRC	73.9 (1.8)	56.4 (2.3)
3.4	Amb	CFC	52.3 (3.7)	65.6 (10.8)
3.4	Amb	HWRC	52.3 (3.2)	49.5 (6.6)
3.4	Dry	CFC	44.9 (10.5)	49.3 (14.1)
3.4	Dry	HWRC	53.6 (10.5)	53.6 (3.6)

Table S3.2. Model comparison of leaf $\delta^{15}N$ with just species and site compared to one with added heat and water treatments.

Model	AIC	BIC	R2_conditional	R2_marginal	ICC	RMSE
Spp*Site*Heat*Water	805.31	1064.46	0.63	0.53	0.21	1.04
Spp + Site + Heat + Water + Site*Heat + Site*Water	869.92	928.66	0.56	0.44	0.22	1.24
Spp * Site	875.43	947.99	0.55	0.41	0.24	1.28
Spp + Site	877.71	922.63	0.53	0.4	0.22	1.3

Table S3.3. Mixed effects model summary for the best model describing the influence of species, site, warming, and rainfall reduction on leaf $\delta^{15}N$ for the fixed effects.

Predictor	**Df**	**Sum Sq.**	**Mean Sq.**	**F value**
Species	8	191.56	23.95	13.14
Site	1	1.04	1.04	0.57
Heat	1	1.64	1.65	0.90
Water	1	26.81	26.81	14.71
Site* Heat	1	4.23	4.23	2.32
Site * Water	1	3.75	3.75	2.06

Table S3.4. Pairwise comparisons of heat and water treatments by site on leaf $\delta^{15}N$.

Contrast	**Site**	**Estimate**	**SE**	**df**	**t-ratio**	**p-value**
Water: Amb - Dry	CFC	-0.44	0.250	181	-1.758	0.0805
	HWRC	-0.95	0.257	186	-3.706	0.0003
Heat: Warm - Amb	CFC	-0.08	0.251	183	-0.331	0.7413
	HWRC	0.48	0.255	182	1.876	0.0623

Table S3.5. Soil $\delta^{15}N$ and soil % N for each site and treatment combination.

Site	Heat	Water	$\delta^{15}N$ Soil ‰ (SD)	Soil % N (SD)	n
CFC	Amb	Amb	0.938 (0.122)	0.17 (0.03)	3
CFC	Warm	Amb	0.893 (0.458)	0.16 (0.02)	3
CFC	Amb	Dry	0.858 (0.721)	0.19 (0.03)	3
CFC	Warm	Dry	1.21 (0.055)	0.19 (0.03)	3
HWRC	Amb	Amb	1.28 (0.185)	0.15 (0.05)	3
HWRC	Warm	Amb	1.82 (0.401)	0.22 (0.15)	3
HWRC	Amb	Dry	1.23 (0.161)	0.15 (0.05)	3
HWRC	Warm	Dry	1.54 (0.04)	0.13 (0.03)	3

Amb = ambient heat or ambient rainfall; Warm = + 3.4°C warming; Dry = ~40% reduced rainfall

Table S3.6. Model comparison for models of concentration of N in soil solution by N form, site, and treatment. The asterisks in the first model indicate that each fixed plus all possible interactions between them were included in the model.

Variables in model	Type	AIC	BIC	R^2	R^2_{adj}	RMSE
N Form * Heat * Water * Site	lm	221.3	277.52	0.49	0.23	0.82
N Form + Site + N Form * Site	lm	194.14	209.88	0.42	0.38	0.88

Table S3.7. ANOVA table for the best fit model of N concentration in soil extracts.

	Df	**Sum Sq.**	**Mean Sq.**	**F-value**	**Pr(>F)**
N Form	2	20.36	10.18	12.13	< 0.0001
Site	1	9.81	9.81	11.69	0.0011
N Form * Site	2	8.78	4.39	5.23	0.0078
Residuals	64	53.73	0.84		

Table S3.8. Model summary for soil solution $\delta^{15}N$ by N form and Site. This table shows the best fit model after backwards model selection that removed both warming and reduced rainfall treatments from the model.

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
N Form	2	5369.8	2684.91	44.98	< 0.0001
Site	1	8.9	8.92	0.15	0.70
N Form * Site	2	317.8	158.88	2.66	0.078
Residuals	64	3820.4	59.69		

Table S3.9. Model estimates for mixed effects model of leaf N by Heat and Water treatment.

Fixed Effects	**Estimate**	**Std. Error**	**t-value**
(Intercept)	0.637	0.056	11.44
Heat: Amb	-0.044	0.024	-1.864
Water: Dry	0.024	0.024	1.025
Random Effects			
Groups	Variance	Std.Dev.	
Site:Spp (Intercept)	0.0485	0.2202	
Residual	0.03276	0.181	

Model Fit: R^2_{cond} = 0.6, R^2_{marg} = 0.008, ICC = 0.6, RMSE = 0.17

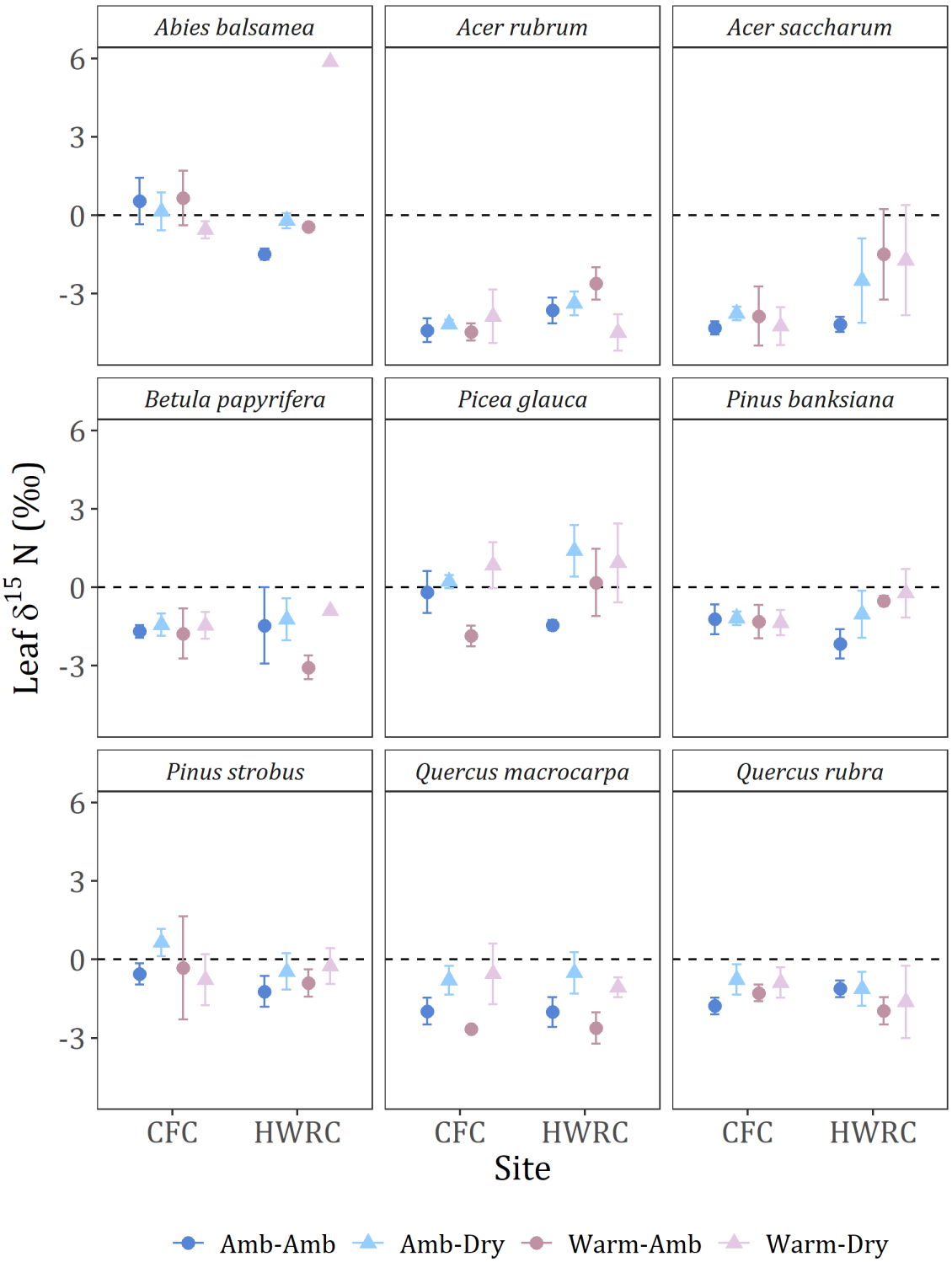

Figure S3.1. Leaf δ^{15}N (mean +/- SE) values by species, site, and treatment.

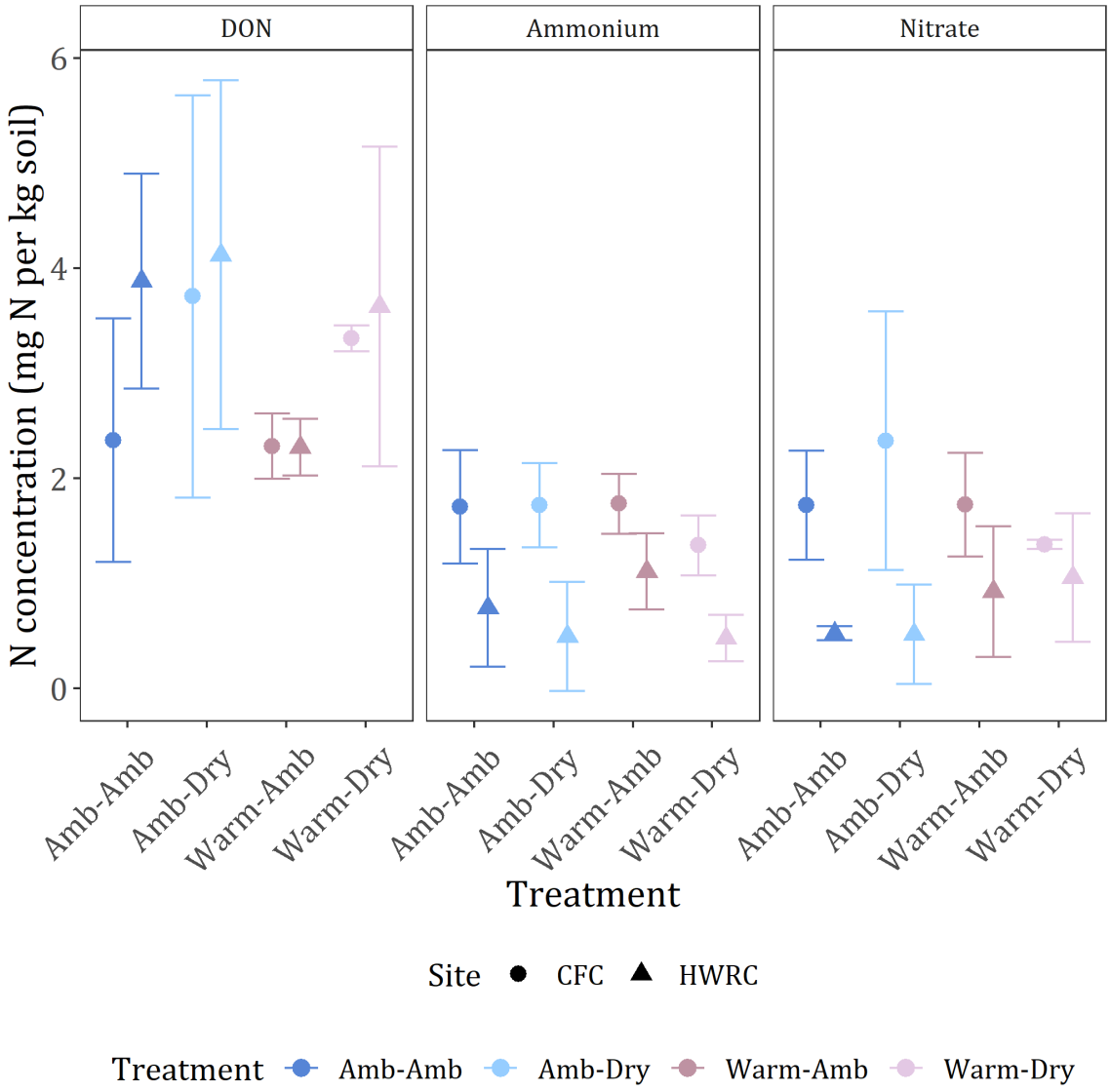

Figure S3.2. Concentrations of DON, ammonium, and nitrate by site and treatment (Mean +/- SE). Concentrations of DON were similar at both sites and tended to be lower in the warm, ambient precipitation plots. Nitrate and ammonium concentrations were higher across treatments at CFC compared to HWRC but did not vary much by treatment.

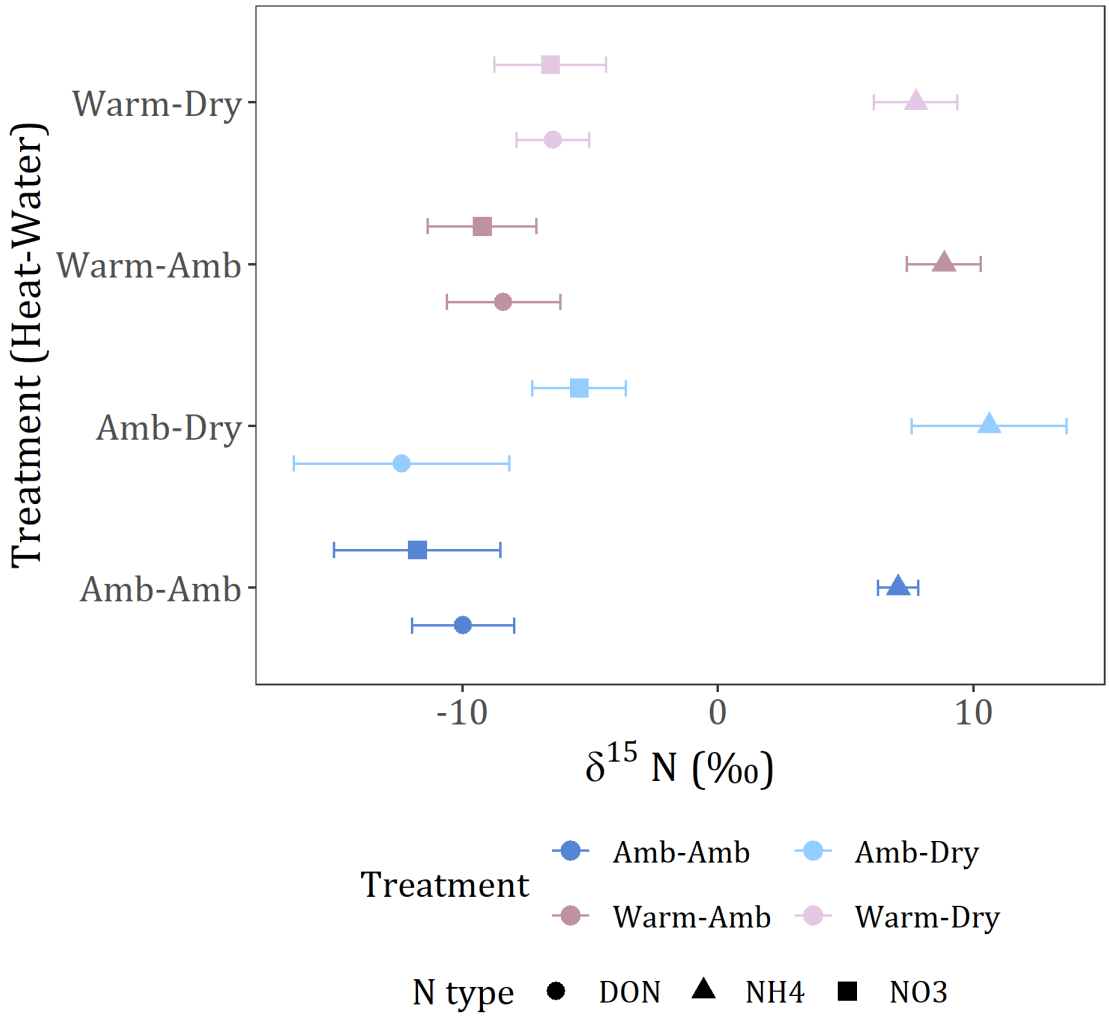

Figure S3.3. Isotopic signatures (mean +/- SE) for each soil N pool by treatment averaged across sites. These values also tend to fall within the range usually reported for terrestrial ecosystems (Hobbie & Högberg, 2012).

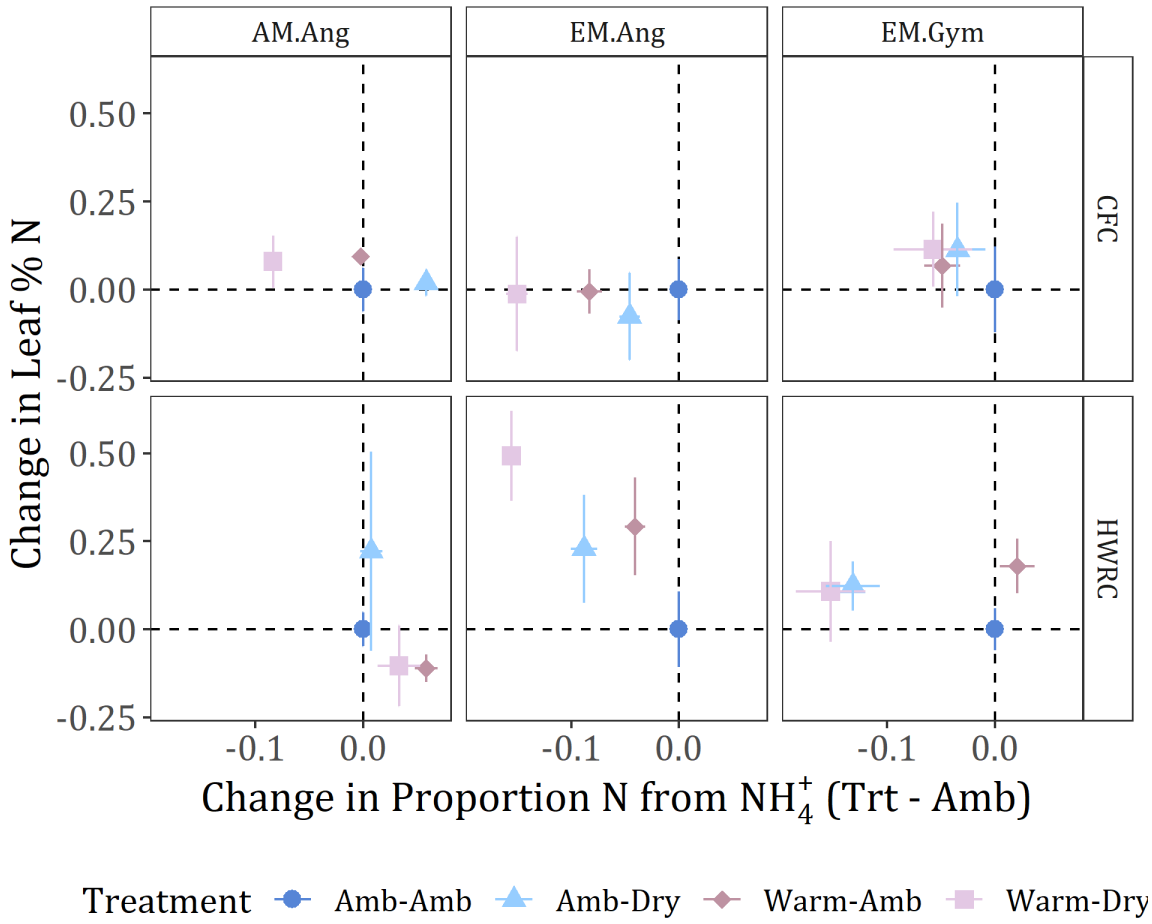

Figure S3.4. Change in NH_4^+ use versus changes in leaf % N by heat and water treatment. Changes are calculated relative to ambient conditions at each site.

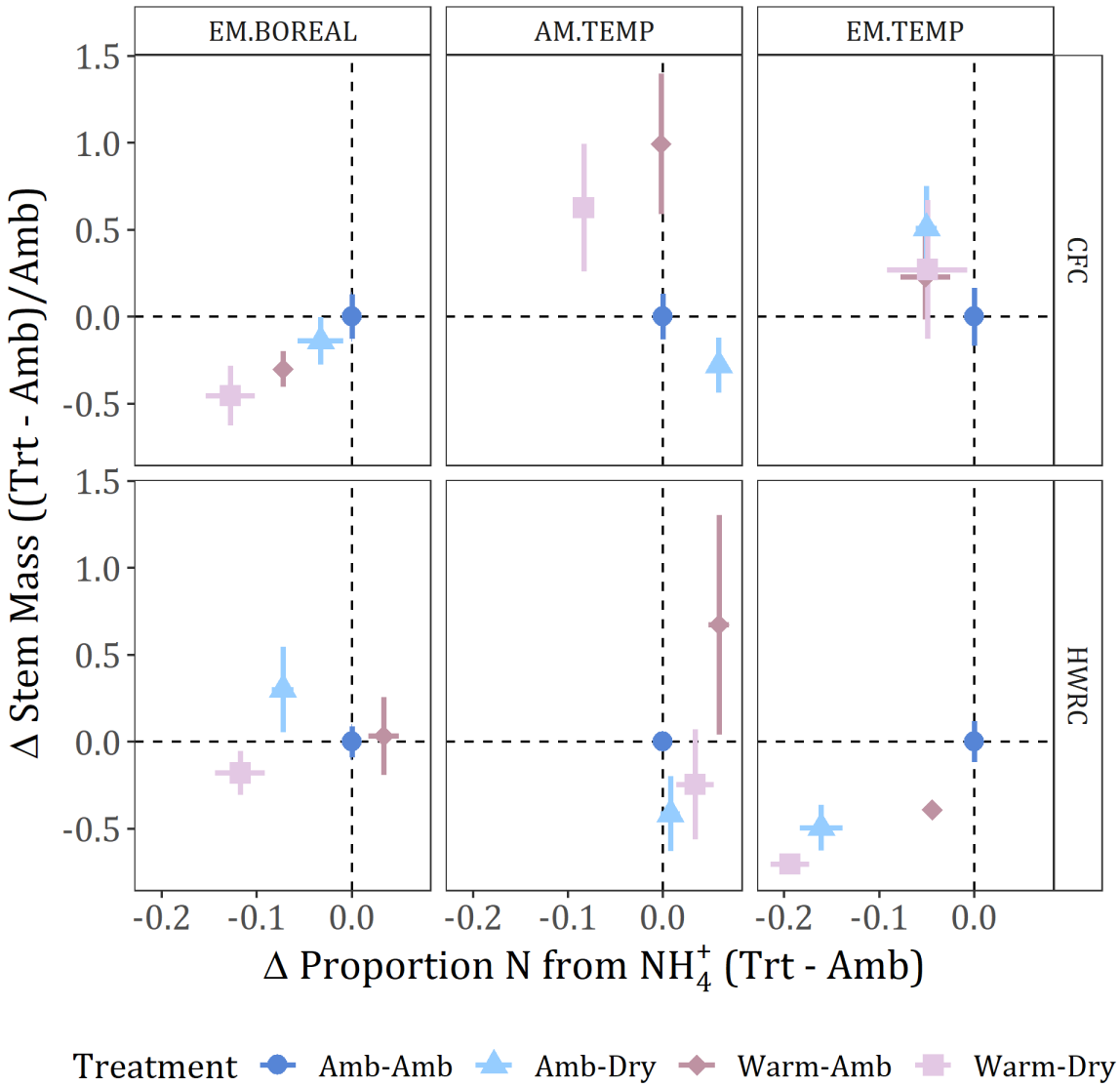

Figure S3.5. Changes in NH_4^+ use compared with changes in stem mass, both relative to the ambient conditions (mean +/- SE). Species are grouped according to MF type and phylogeny as well as by site.

APPENDIX 4 – CHAPTER 4 SUPPLEMENTAL MATERIALS

Supporting Methods

Detecting and dealing with outliers

For all numeric variables, we identified potential outliers as those points with a chi-square value > 95% of values. We then made Cleveland Dot Plots of all variables with potential outliers to visually assess whether outliers should be removed (isolated points on the left or right side of the plot) (Zuur *et al.*, 2007). For C and N soil data, we also made bivariate plots of C vs. N content to help assess whether an outlier looked like an error (i.e. not falling in the 95% confidence ellipse) since these two variables are usually correlated. Based on these plots, we identified 8 data points as outliers in 4 variables (potential N min (3), DIN (1), soil N % (3), soil C % (1)): for the outliers in %C and %N, we used the relationship between %C and %N to calculate the missing C or N data (see equation below); for other variables we calculated the species-level mean and used those values for analysis. When needed, variables were log-transformed prior to statistical analysis.

$$\text{Total N} = \exp(\ln(\text{Total C}) * 0.331 - 2.609)$$

<u>Supporting results</u>

Correlations between N cycling metrics

In terms of correlations between resin N accumulation, potential N cycling rates, and in situ N cycling rates, there were 153 possible correlations, of which 43 (~28%) were strong ($|r| > 0.4$ and $p < 0.05$). *In situ* N mineralization and nitrification rates were

both strongly correlated with potential nitrification and potential N mineralization in the A horizon ($r > 0.5$ and $p < 0.0001$ for all). In addition to the correlations with in situ mineralization and nitrification, potential N mineralization and potential nitrification were strongly correlated with each other in the A horizon ($r = 0.77$, $p < 0.0001$), and potential nitrification in the O horizon was strongly correlated with resin NO_3^- accumulation in the A horizon ($r = 0.53$, $p < 0.0001$). The resin N accumulation data were correlated with each other, even across horizons, with the strongest correlation between NH_4^+ accumulation in the O and A horizons ($r = 0.81$, $p < 0.0001$). However, aside from the previously mentioned correlation between potential nitrification in the O horizon and A horizon resin NO_3^-, resin N accumulation was not strongly correlated with the other metrics of N cycling.

Table S4.1. Summary of studies citing Phillips et al. (2013) as of May 2018. Criteria for each paper to be counted in each category is as follows: *Contrasts AM and EM species:* includes at least one AM and one EM species; *Primary data paper:* includes field or laboratory measurements (e.g. not a review, meta-analysis, or commentary); *Includes gymnosperms*: includes at least one gymnosperm; *Study Biome: Temperate forest:* study site characterized as temperate forest; *Study Location: Europe*: study site was located in Europe or had primarily European species if in a greenhouse; *Study focus: carbon cycling:* study includes some metrics of carbon cycling or storage in soils

Criteria												
Cites Phillips et al. (2013)	●	●	●	●	●	●	●	●	●	●	●	●
Contrasts AM and EM species		●						●	●	●	●	●
Experimental Study			●					●	●	●	●	●
Includes gymnosperms				●					●	●	●	●
Study Biome: Temperate forest					●					●	●	●
Study Location: Europe						●					●	●
Study focus: carbon cycling							●					●
Total # of studies	147	85	91	85	61	52	31	58	33	27	3	2
Percent of Citing Studies	100%	58%	62%	58%	41%	35%	21%	39%	22%	18%	2%	1%

Table S4.2. AIC and BIC values for comparing models fit with MF type + Phylogeny, just MF type, and just Phylogeny.

	MF+Phy		**MF**		**Phy**		**MF+Phy vs. MF**		**MF+Phy vs. Phy**		**MF vs. Phy**	
	AIC	BIC	AIC	BIC	AIC	BIC	ΔAIC	ΔBIC	ΔAIC	ΔBIC	ΔAIC	ΔBIC
Leaf Decomposition (Home Plot)	-66.1	-56.2	-67	-59.1	-66.4	-58.5	0.9	2.9	0.3	2.3	-0.6	-0.6
Root Decomposition (Common Plot)	-73.8	-65.4	-71	-64.2	-74.3	-67.5	-2.8	-1.2	0.5	2.1	3.3	3.3
Forest Floor Turnover	45.3	55.2	51.1	59	51.6	59.5	-5.8	-3.8	-6.3	-4.3	-0.5	-0.5
Root Ingrowth	-4	5.85	-5.99	1.89	1.88	9.76	1.99	3.96	-5.88	-3.91	-7.87	-7.87
Litterfall	-34.17	-24.33	-31.73	-23.84	-36.1	-28.22	-2.44	-0.49	1.93	3.89	4.37	4.38
Total Inputs	-55.34	-45.48	-52.84	-44.96	-56.02	-48.14	-2.5	-0.52	0.68	2.66	3.18	3.18
Net N mineralization	-2.37	7.48	-4.37	3.51	0.27	8.16	2	3.97	-2.64	-0.68	-4.64	-4.65
Net nitrification	107.32	117.17	107.38	115.26	110.58	118.46	-0.06	1.91	-3.26	-1.29	-3.2	-3.2
DON:DIN	12.65	22.4	24.33	32.13	11.1	18.89	-11.68	-9.73	1.55	3.51	13.23	13.24
Total C (O + 75 cm min)	-76.5	-66.65	-73.64	-65.76	-77.41	-69.53	-2.86	-0.89	0.91	2.88	3.77	3.77
Total C (O horizon)	35.9	45.75	37.59	45.47	40.09	47.97	-1.69	0.28	-4.19	-2.22	-2.5	-2.5
Prop C in top 10cm (O + 10cm min)	-56.14	-46.28	-57.53	-49.65	-56.66	-48.78	1.39	3.37	0.52	2.5	-0.87	-0.87
DOC (A Horizon)	-5.91	3.94	-5.89	1.99	-6.69	1.18	-0.02	1.95	0.78	2.76	0.8	0.81
DOC (O horizon)	-12.72	-2.87	-14.6	-6.72	-13.88	-6	1.88	3.85	1.16	3.13	-0.72	-0.72
Total N (O + 75 cm min)	-58.56	-50.68	-60.52	-54.61	-60.56	-54.65	1.96	3.93	2	3.97	0.04	0.04
Total N O Horizon	41.51	51.36	42.74	50.62	46.21	54.09	-1.23	0.74	-4.7	-2.73	-3.47	-3.47
C:N - O horizon	-134.91	-125.06	-136.43	-128.55	-135.95	-128.07	1.52	3.49	1.04	3.01	-0.48	-0.48
C:N - 10 cm	279.19	289.04	279.32	287.2	283.77	291.65	-0.13	1.84	-4.58	-2.61	-4.45	-4.45
C:N – Whole Profile	-53.77	-43.92	-49.44	-41.56	-54.63	-46.75	-4.33	-2.36	0.86	2.83	5.19	5.19

Table S4.3. Model fits for additional pools, fluxes, and plant traits. In cases where the LM fit the data better but variance for random effects could still be estimated, we present the results for the LMM. In cases where multiple models had AIC and BIC values within 2, we present the model with the lowest AIC value first followed by the next model(s) in parentheses.

Property/Dependent Variable	Best Model Type	Best Model(s)	R2*	ICC	RMSE
Leaf Lignin:N	LMM	Phy	0.70, 0.15	0.65	0.19
Leaf P	LMM	Phy	0.74, 0.32	0.61	0.08
Leaf C:N	LMM	Phy	0.68, 0.17	0.62	0.08
Root Lignin:N	LMM	Phy	0.64, 0.23	0.54	0.07
Root P	LMM	(Phy)	0.52, 0.02	0.51	0.06
Root C:N	LMM	Phy	0.53, 0.16	0.44	0.07
Resin NH_4^+	LMM	Phy*horizon	0.72, 0.62	0.28	0.19
Resin NO_3^-	LMM	Phy*horizon	0.77, 0.67	0.29	0.43
Potential N min	LMM	Phy (MF) + horizon	0.37, 0.09	0.31	0.34
Potential Nit	LM (LMM)	Phy (MF+Phy) + horizon	0.19, 0.10	0.1	0.47
Total C (75 cm mineral)	LM**	Phy	0.02		0.14
Top 10 cm C (mineral)	LMM (LM)	Phy	0.24, 0.04	0.21	0.15
MBC	LM (LMM)	MF+Phy * horizon (Phy, MF)	0.20, 0.19	0.01	0.27
MBC to MBN	LM**	MF+Phy + horizon	0.02		0.13
Fungal:Bacterial PLFA	LM	MF+Phy	0.35, 0.26	0.12	0.13
soil pH (saturated paste)	LM**	MF+Phy	0.33		0.44
soil pH water	LM (LMM)	MF+Phy (Phy)	0.48, 0.34	0.21	0.01
CEC	LM**	MF (MF+Phy)	0.23		0.16
Base Sat	LMM (LM)	MF	0.38, 0.20	0.22	0.23
Clay	LM	MF	0.35, 0.34	0.004	0.22
L. terrestris biomass	LMM	MF	0.63, 0.16	0.56	0.14
Net N Mineralization Rate	LMM (LM)	Phy + avesoilmoist	0.44, 0.30	0.2	0.16
Net Nitrification	LM	Phy (MF) + Fungal:Bacterial	0.47		0.46
N (top 10 cm + O)	LMM	Phy (MF)	0.48, 0.05	0.46	0.12
DON:DIN + EM richness	LM (LMM)	Phy + EM rich	0.43, 0.38	0.08	0.22

*for LMMs, the first value is R^2 conditional (variation explained by fixed and random effects) and the second is R^2 marginal (variation explained by fixed effects only); for LM, the adjusted R^2 is presented; **indicates LM used because of singular fit w/random effect

Abbreviations: LMM = Linear Mixed-Effects Model; LM = Linear Model; ICC = Intraclass correlation coefficient (higher values = greater proportion of variance explained by the grouping variable)

Table S4.4. Model estimates for additional analyses of pools, fluxes, and plant traits. Continued on the following page.

Property/Dependent Variable	Contrast	Significance test
Leaf Lignin:N	Ang – Gym	t = -1.774, p = 0.102, df = 11.9
Leaf C:N	Ang – Gym	t = -1.916, p = 0.080, df = 11.9
Leaf P	Ang – Gym	t = 2.95, p = 0.012, df = 11.9
Root Lignin:N	Ang – Gym	t = 2.41, p = 0.033, df = 11.8
Root C:N	Ang – Gym	t = 11.6, p = 0.060, df = 11.6
Root P	Ang – Gym	t = 0.587, p = 0.568, df = 11.7
N mineralization + soil moisture	Ang – Gym	t = -0.562, p =0.58, df = 11.9
Nitrification + fungal:bacterial	Ang – Gym	t = 0.885, df = 50, p = 0.38
DON:DIN + EM richness	Ang – Gym	t = 1.70, p = 0.10, df = 21.4
Resin NH_4^+	O: Ang – Gym	t = -3.77, p = 0.0014, df = 17.9
	A: Ang – Gym	t = -5.23, p = 0.0001, df = 17.9
	Ang: A – O	t = 6.92, p < 0.0001, df = 90.1
	Gym: A – O	t = 9.11, p < 0.0001, df = 90.1
Resin NO_3^-	O: Ang – Gym	t = -3.19, p = 0.0051, df = 17.7
	A: Ang – Gym	t = -5.84, p < 0.0001, df = 17.7
	Ang: A – O	t = 7.54, p < 0.0001, df = 90.1
	Gym: A – O	t = 11.69, p < 0.0001, df = 90.1
Potential N mineralization	Ang – Gym	t = -0.79, p = 0.44, df = 11.7
	A – O	t = -3.47, p = 0.0008, df = 91.1
Potential Nitrification	Ang – Gym	t = -2.49, p = 0.03, df = 11.1
	A – O	t = 0.52, p = 0.6, df = 91.3
Total C (75 cm min)	Ang – Gym	t = -1.48, df = 51, p = 0.145
Top 10 cm C	Ang – Gym	t = -1.15, p = 0.28, df = 11.2
MBC (mg per g C)	O: AM ang – EM ang	t = 1.73, p =0.20, df = 48.2
	O: AM ang – EM gym	t = 3.72, p = 0.0016, df = 45.3
	O: EM ang – EM gym	t = 2.96, p = 0.015, df = 32
	A: AM ang – EM ang	t = 1.69, p = 0.22, df = 48.2
	A: AM ang – EM gym	t = 2.34, p = 0.06, df = 44.7
	A: EM ang – EM gym	t = 0.937, p = 0.62, df = 30.9
MBC to MBN	AM ang – EM ang	t = -0.654, p = 0.79, df = 101
	AM ang – EM gym	t = -1.62, p = 0.24, df = 101
	EM ang – EM gym	t = -1.47, p = 0.31, df = 101
Top 10cm N w/O	Ang – Gym	t = -1.025, p = 0.326, df = 11.7
FtoB ratio (PLFA)	AM ang – EM ang	t = -3.36, p = 0.012, df = 13.93
	AM ang – EM gym	t = -1.51, p = 0.32, df = 12.99
	EM ang – EM gym	t = 2.79, p = 0.047, df = 9.96
soil pH Sat paste	AM ang – EM ang	t = 2.82, p = 0.019, df = 50
	AM ang – EM gym	t = 4.92, p < 0.0001, df = 50
	EM ang – EM gym	t = 3.149, p = 0.0077, df = 50
soil pH H_2O 20 cm	AM ang – EM ang	t = 1.85, p = 0.19, df = 12.9
	AM ang – EM gym	t = 3.788, p = 0.0065, df = 12.3
	EM ang – EM gym	t = 2.795, df = 10.4, p = 0.045

Property/Dependent Variable	Contrast	Significance test
CEC	AM – EM	t = 4.098, p = 0.0001, df = 51
Percent Clay	AM – EM	t = 5.121, p = 0.0001, df = 18
Base Sat	AM – EM	t = 2.99, p = 0.010, df = 14.2
L. terrestris biomass	AM – EM	t = 2.133, p = 0.053, df = 12.6

(Table continued from previous page)

Table S4.5. Correlations between variables and the principle components 1-3.

Variable*	Dim.1	Dim.2	Dim.3
Potential Nitrification (A horizon)	-0.296	0.611	-0.115
Potential Nitrification (O horizon)	-0.382	0.407	0.008
Potential N mineralization (A horizion)	-0.120	0.808	-0.237
Potential N mineralization (O horizon)	-0.434	0.175	-0.413
Root decomposition (Common Litter)	0.477	-0.048	-0.153
Soil C (O horizon)	-0.729	-0.557	-0.007
Soil C (Whole Profile)	-0.597	-0.106	0.164
Soil C:N (O horizon)	0.149	-0.013	0.477
Soil C:N (Whole Profile)	-0.459	-0.258	0.545
DOC (A horizon)	-0.311	-0.059	0.114
DOC (O horizon)	0.052	-0.638	-0.237
DON:DIN	0.642	0.000	-0.366
Forest Floor Turnover	0.809	0.432	0.217
Leaf litter *k* (Home Plot)	0.388	-0.298	0.078
Fine root ingrowth	0.301	0.369	0.212
Leaf litterfall	0.575	-0.175	0.486
MBC (A horizon)	0.206	0.554	-0.152
MBC (O horizon)	0.477	-0.257	0.415
Soil N (O horizon)	-0.731	-0.539	-0.080
Soil N (Whole Profile)	-0.049	0.193	-0.463
NH4 Resin (A horizon)	-0.766	0.115	0.272
NH4 Resin (O horizon)	-0.839	0.250	0.229
Nitrification rate	0.003	0.757	0.235
N mineralization rate	0.090	0.687	0.221
NO3 Resin (A horizon)	-0.799	0.201	0.200
NO3 Resin (O horizon)	-0.681	0.316	0.303
Total Inputs (Leaf + root)	0.627	-0.079	0.537

Table S4.6. AIC and BIC values for additional models of pools, fluxes and plant traits.

	MF+Phy		MF		Phy		MF+Phy vs. MF		MF+Phy vs. Phy		MF vs. Phy	
	AIC	BIC	AIC	BIC	AIC	BIC	ΔAIC	ΔBIC	ΔAIC	ΔBIC	ΔAIC	ΔBIC
Leaf Lignin:N	-64.28	-54.43	-64.26	-56.38	-65.06	-57.18	-0.02	1.95	0.78	2.75	0.8	0.8
Leaf C:N	-74.81	-64.96	-71.76	-63.88	-75.41	-67.53	-3.05	-1.08	0.6	2.57	3.65	3.65
Leaf P	-68.54	-58.69	-61.44	-53.56	-69.12	-61.24	-7.1	-5.13	0.58	2.55	7.68	7.68
Root Lignin:N	-83.92	-74.16	-80.61	-72.9	-85.85	-78.04	-3.31	-1.26	1.93	3.88	5.24	5.14
Root CN	-91.96	-82.21	-89.37	-81.56	-93.63	-85.82	-2.59	-0.65	1.67	3.61	4.26	4.26
Root P	-97.4	-87.64	-98.97	-91.17	-99.37	-91.56	1.57	3.53	1.97	3.92	0.4	0.39
Resin NH4	170.66	191.97	183.33	199.31	166.92	182.9	-12.67	-7.34	3.74	9.07	16.41	16.41
Resin NO3	180.85	199.49	230.97	244.28	178.79	192.11	-50.12	-44.79	2.06	7.38	52.18	52.17
Potential N min.	115.41	131.39	114.31	127.63	113.6	126.92	1.1	3.76	1.81	4.47	0.71	0.71
Potential Nit.	162.75	178.74	169.28	182.59	163.93	177.25	-6.53	-3.85	-1.18	1.49	5.35	5.34
Total C (75 cm min)	-49.94	-42.06	-49.63	-43.72	-51.82	-45.91	-0.31	1.66	1.88	3.85	2.19	2.19
Top 10 cm C (mineral)	-28.05	-18.2	-28.83	-20.95	-30.03	-22.15	0.78	2.75	1.98	3.95	1.2	1.2
MBC	40.42	61.65	45.38	61.3	42.38	58.3	-4.96	0.35	-1.96	3.35	3	3
MBC to MBN	-126.48	-113.21	-126.26	-115.65	-128.04	-117.42	-0.22	2.44	1.56	4.21	1.78	1.77
Fungal:Bacterial PLFA	-48.69	-38.84	-42.93	-35.05	-40.28	-32.4	-5.76	-3.79	-8.41	-6.44	-2.65	-2.65
soil pH (sat. paste)	-181.33	-173.45	-173.75	-167.83	-175.53	-169.62	-7.58	-5.62	-5.8	-3.83	1.78	1.79
soil pH water	-266.57	-256.72	-261.05	-253.17	-264.81	-256.93	-5.52	-3.55	-1.76	0.21	3.76	3.76
CEC	-34.05	-26.17	-35.29	-29.38	-20.53	-14.62	1.24	3.21	-13.52	-11.55	-14.76	-14.76
Base Sat	19.21	29.06	17.5	25.38	23.61	31.49	1.71	3.68	-4.4	-2.43	-6.11	-6.11
Clay	-1.49	8.27	-3.45	4.36	13.31	21.11	1.96	3.91	-14.8	-12.84	-16.76	-16.75
Earthworm biomass	-16.68	-6.83	-17.5	-9.62	-15.5	-7.62	0.82	2.79	-1.18	0.79	-2	-2
Net N Min + soil moisture	-14.18	-2.36	-15.68	-5.83	-15.97	-6.12	1.5	3.47	1.79	3.76	0.29	0.29
Net Nit. + Fungal:Bacterial	76.8	86.65	74.85	82.73	75.62	83.5	1.95	3.92	1.18	3.15	-0.77	-0.77
N (top 10 cm + O)	-38.49	-28.64	-39.26	-31.38	-40.43	-32.55	0.77	2.74	1.94	3.91	1.17	1.17
DON:DIN + EM richness	12.65	22.4	24.33	32.13	11.09	18.89	-11.68	-9.73	1.56	3.51	13.24	13.24

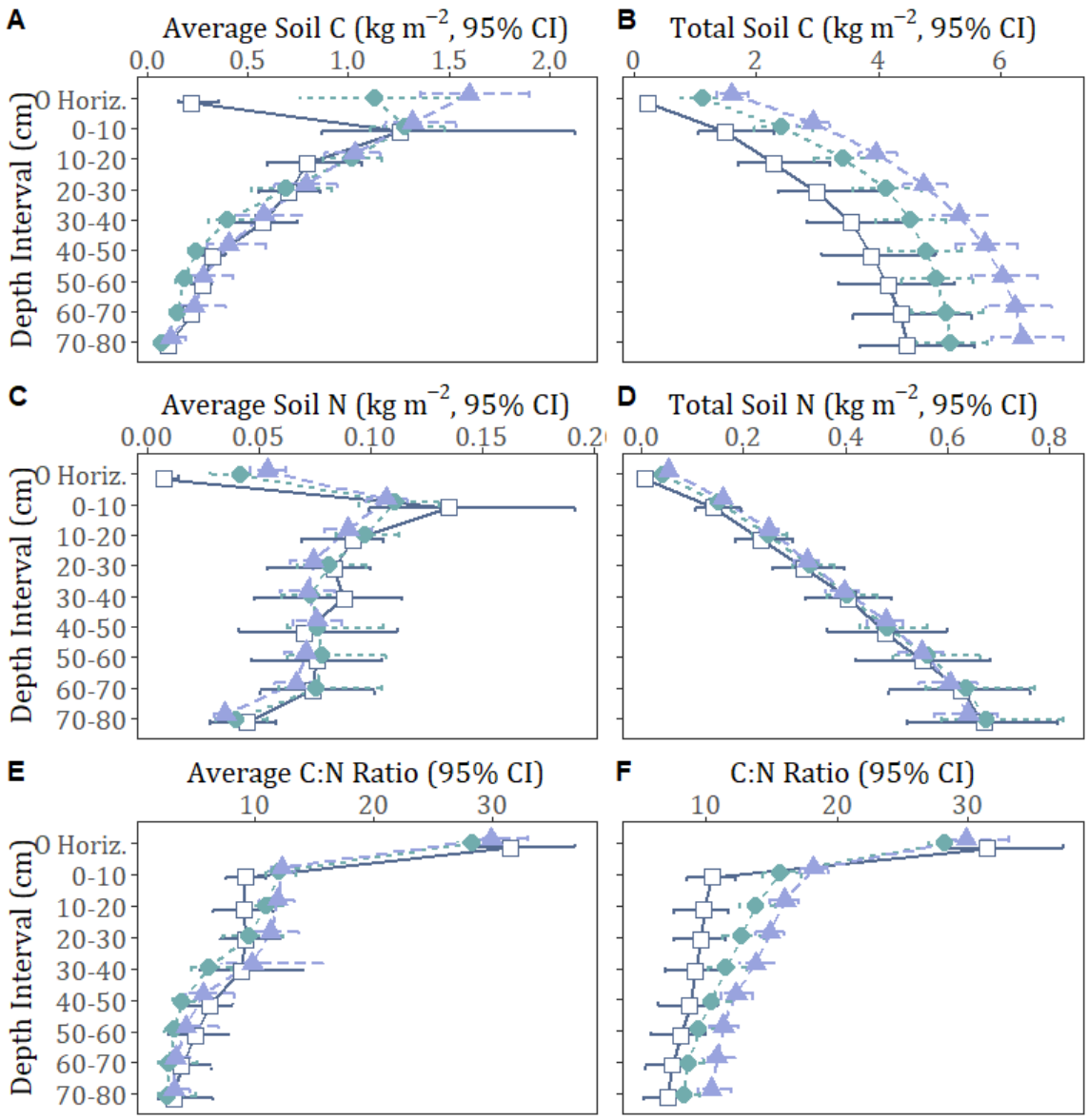

Figure S4.1. Total soil carbon, nitrogen, and C:N ratios by mycorrhizal type and phylogeny shown in 10 cm depth increments. Plots A, C, E show the mean for each 10cm interval and plots B, D, F show the cumulative mean as you move down the profile (i.e. the top point is just the O horizon soil C, the second point includes soil C in the O horizon and the top 10 cm of mineral soil, etc.). Open squares: AM angiosperms; Closed circles: EM Angiosperms; Closed triangles: EM Gymnosperms.

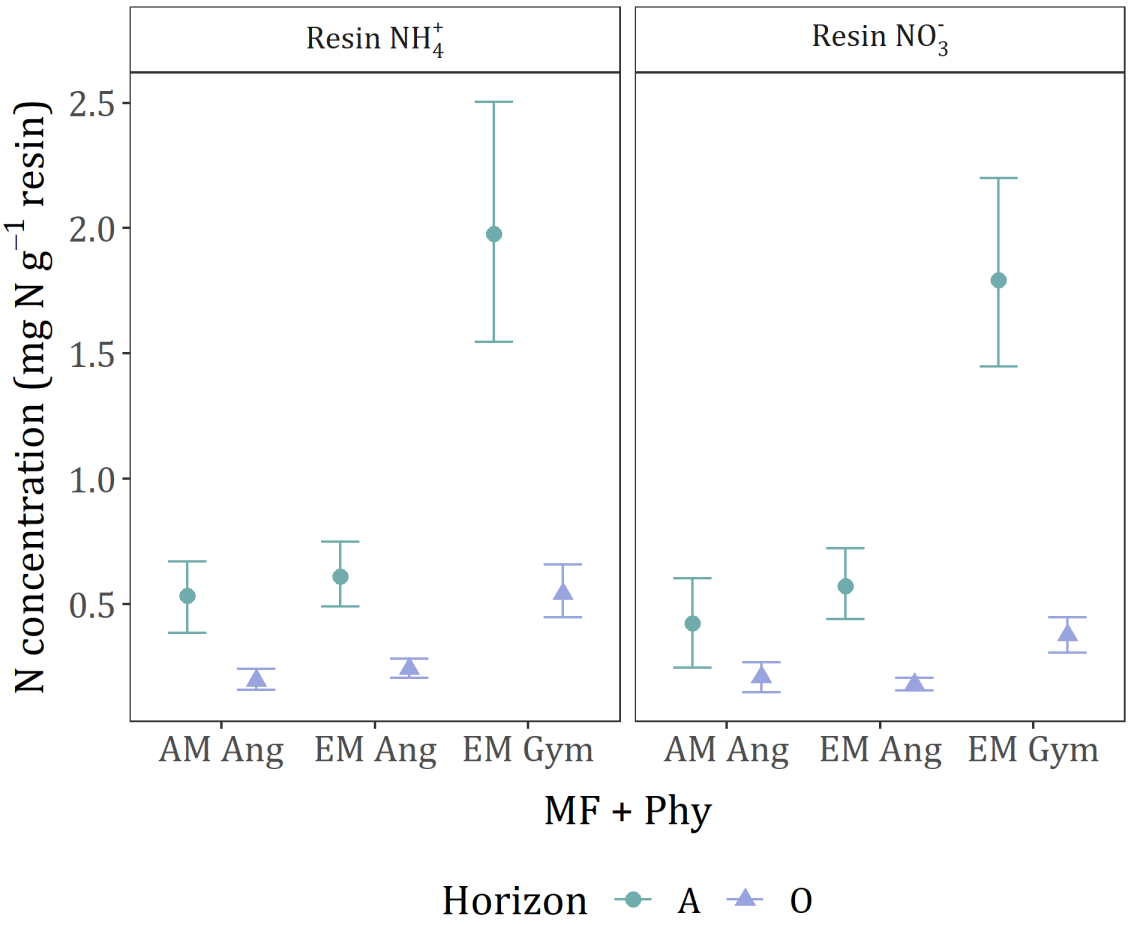

Figure S4.2. Accumulation of nitrogen as NH_4^+ and NO_3^- on resin bags in the O and A horizons by mycorrhizal type and phylogeny. Both plots have the same y-axis scale and error bars represent the 95% CI around the mean.

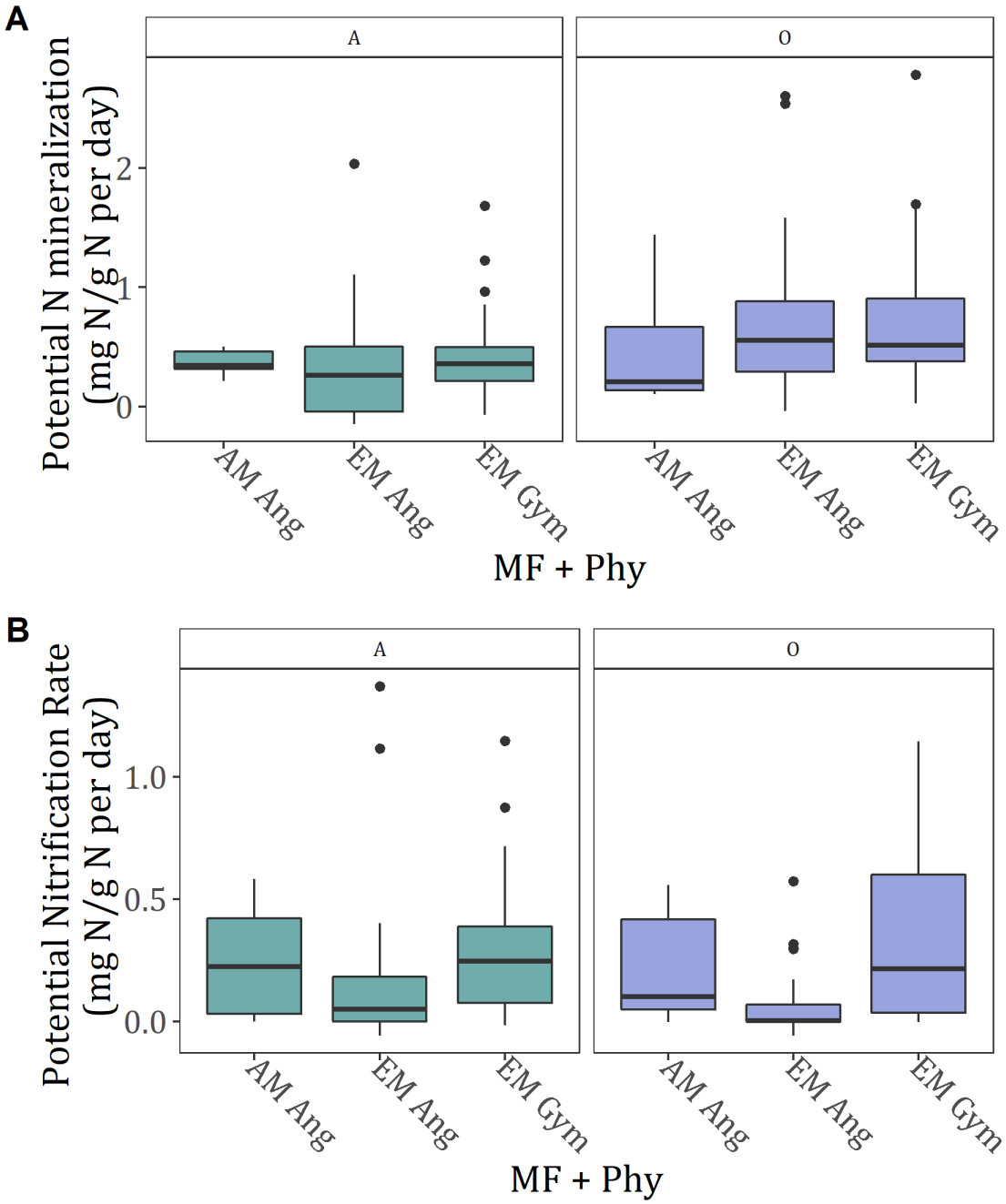

Figure S4.3. Potential N mineralization and nitrification rates by mycorrhizal type and phylogeny for the A and O horizons. For both plots A and B the left panel shows rates for the A horizon and the right plot shows rates for the O horizon. Neither mycorrhizal type or phylogeny explained much variation in potential rates of N mineralization or nitrification in the short-term incubations.

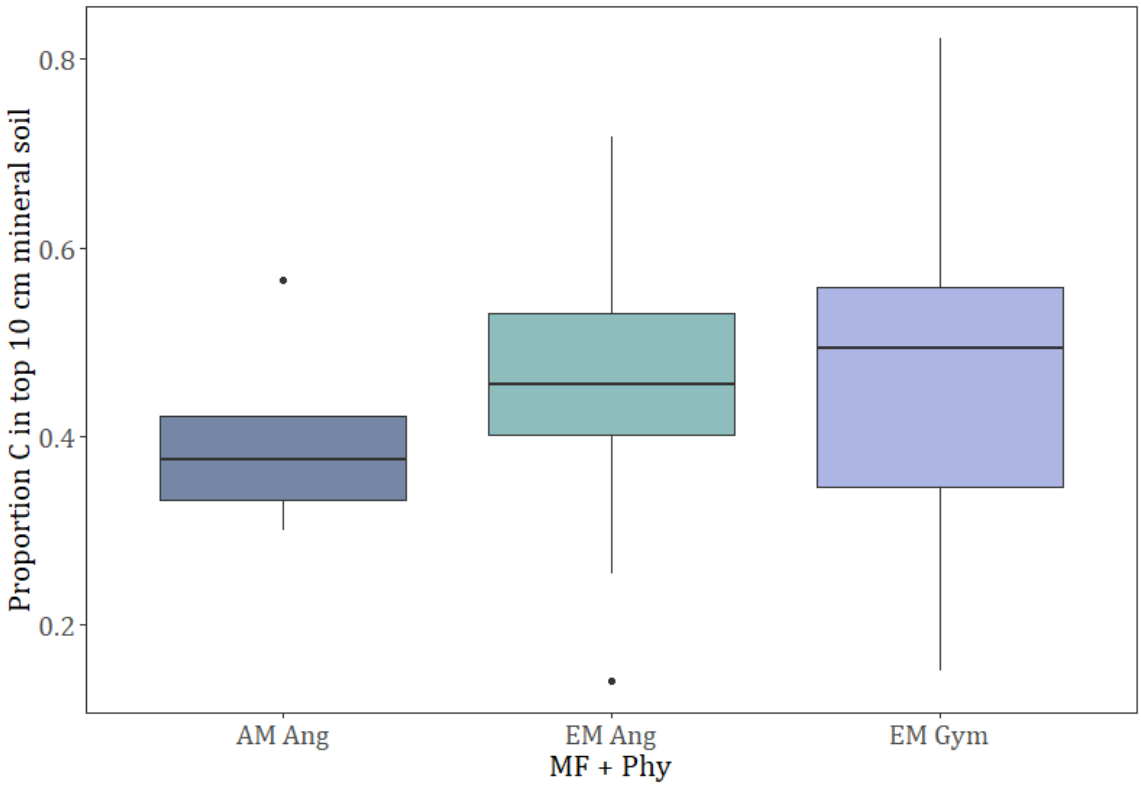

Figure S4.4. Proportion of total soil C stored in the top 10 cm of soil (excluding O horizon) by mycorrhizal type and phylogeny. EM angiosperm and gymnosperm plots had a trend toward storing a greater proportion of their C in the top 10 cm of soil compared to AM angiosperms.

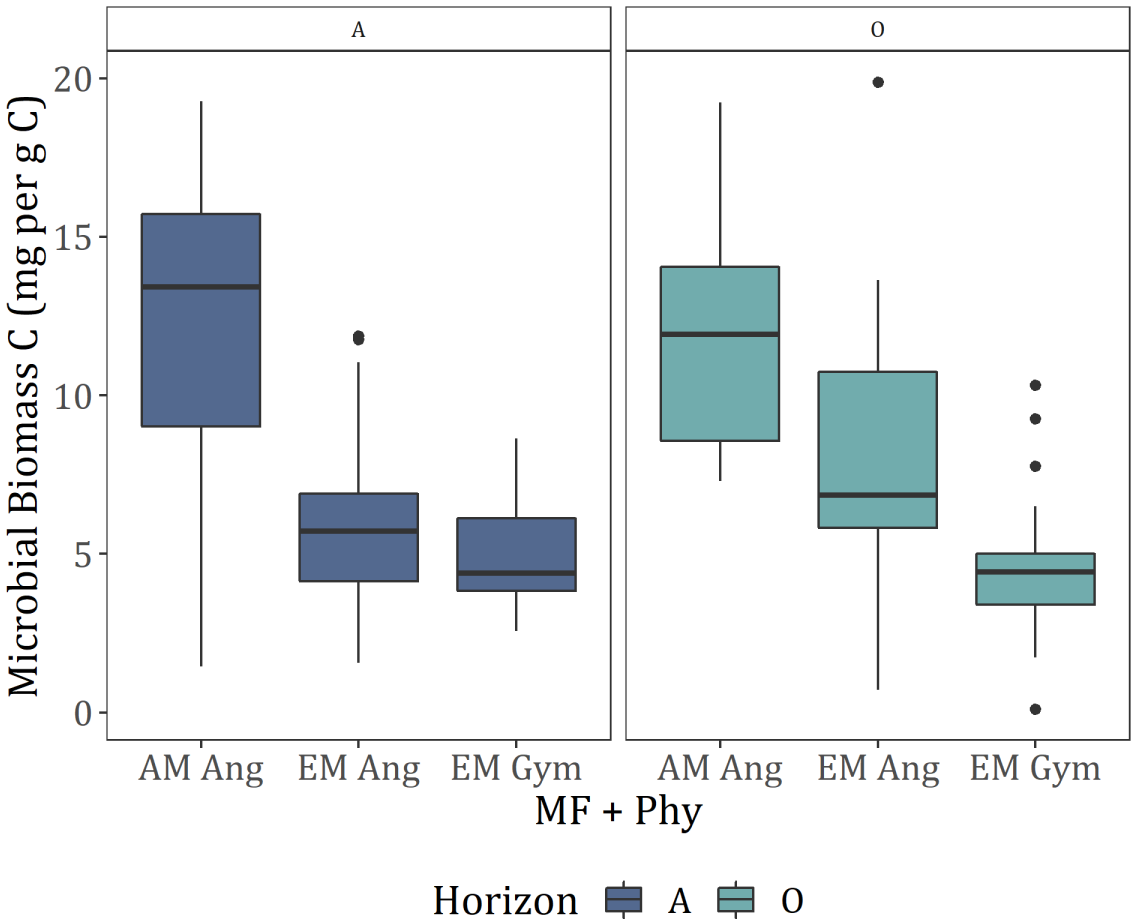

Figure S4.5. Microbial biomass carbon by horizon (O vs. A), mycorrhizal type, and phylogeny. The left panel shows microbial biomass carbon for the A horizon and the right panel shows microbial biomass carbon for the O horizon.

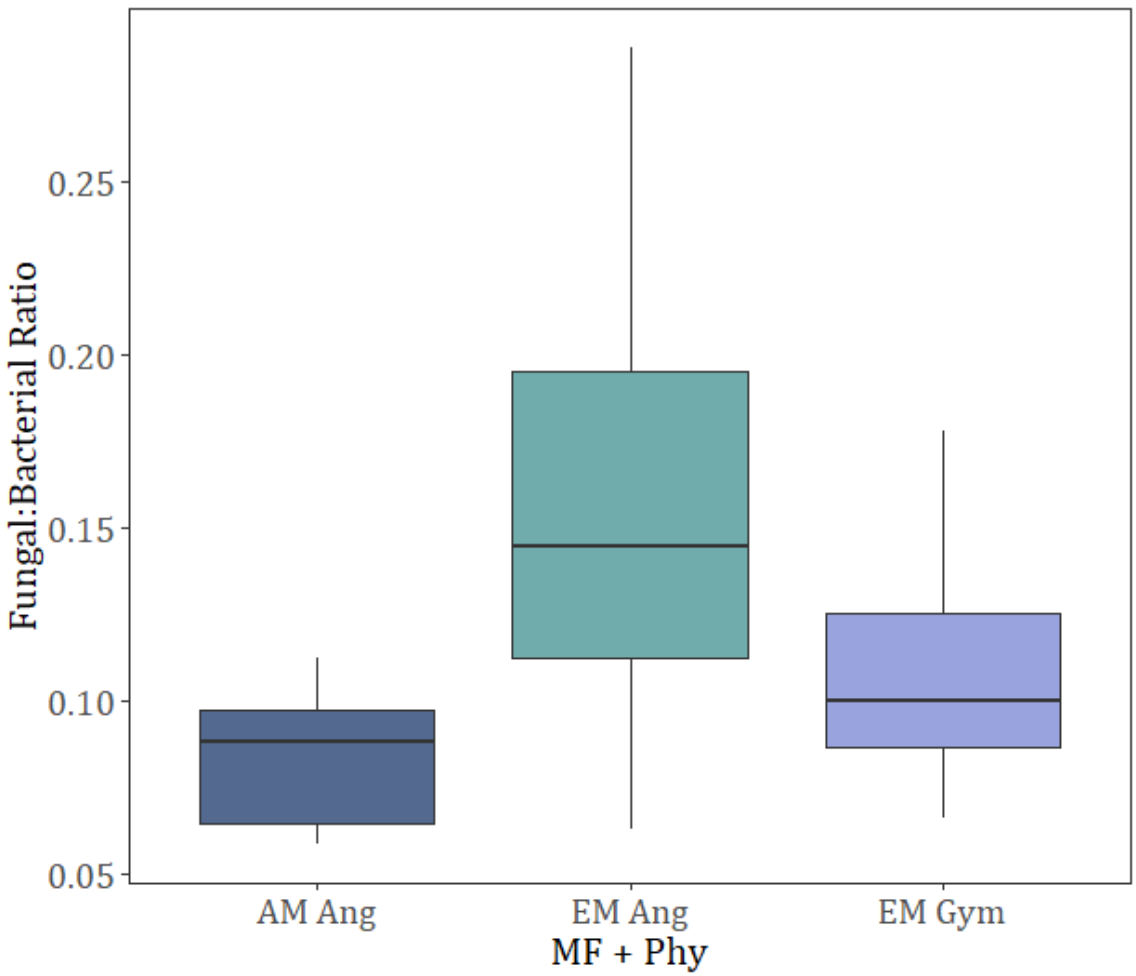

Figure S4.6. Microbial community composition by mycorrhizal type and phylogeny. All three groups differed in the ratio of fungi:bacteria ($F_{2,50}$ = 9.022, p = 0.000451).

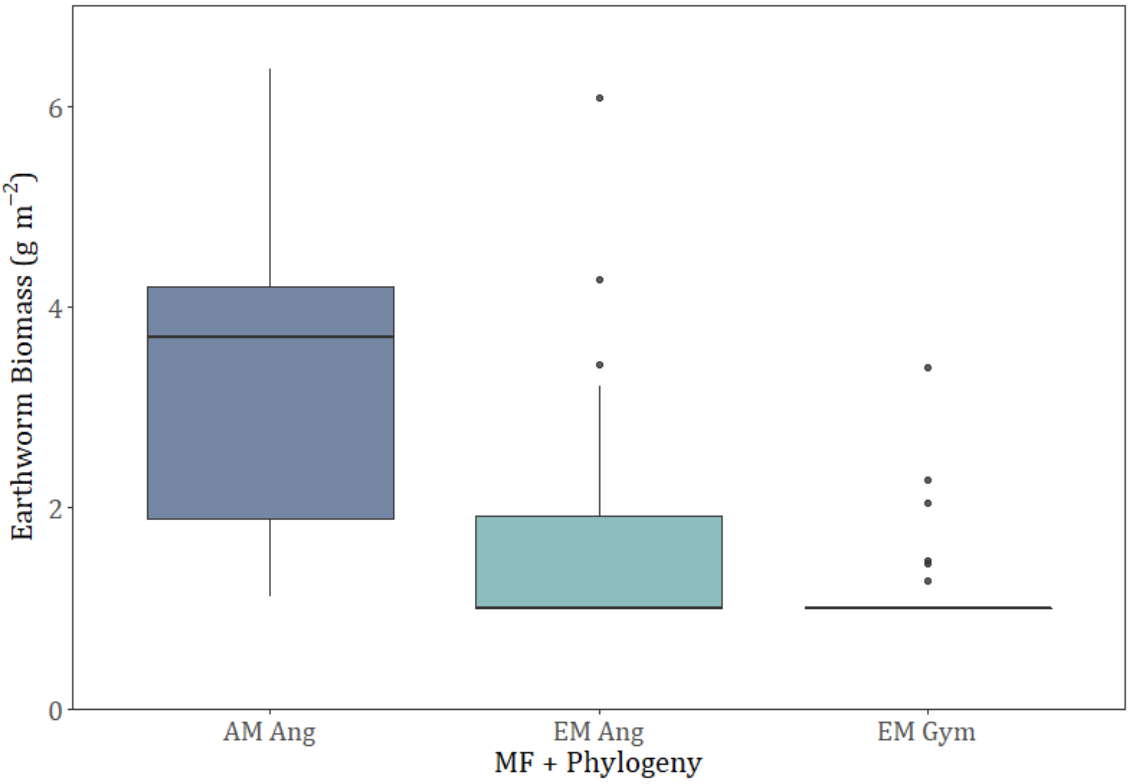

Figure S4.7. Average earthworm biomass (*L. terrestris*) in plots by mycorrhizal type and phylogeny.