MYCORRHIZAL TYPE AND LITTER STOICHIOMETRY DETERMINE SOIL BIOGEOCHEMISTRY IN A TEMPERATE FOREST

by

MELANIE KAY TAYLOR

(Under the Direction of Nina Wurzburger)

ABSTRACT

Mycorrhizal symbioses affect soil biogeochemistry across terrestrial ecosystems. The dominance of arbuscular mycorrhizal (AM) plant species is associated with lower soil carbon (C) to nitrogen (N) ratios relative to ectomycorrhizal (ECM) plant species, but it is unclear how these patterns have emerged. Explanations include mycorrhizal differences in nutrient acquisition strategies, plant litter stoichiometry, or a combination. We tested these ideas in a mixed-mycorrhiza temperate forest by pairing a field study on tree-specific soil characteristics with a soil respiration mesocosm experiment where we manipulated soils and litters. Our results demonstrate that both mycorrhizal type and litter stoichiometry determine patterns in soil biogeochemistry with litter stoichiometry and mycorrhizal type affecting soil C:N and the heterotrophic respiration response. Our study demonstrates that the mycorrhizal type and litter stoichiometry of tree species create distinct biogeochemical signatures on soils in this forest and creates a predictive context for considering the consequences of tree species migration.

INDEX WORDS: mycorrhiza; biogeochemistry; litter stoichiometry; decomposition

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MELANIE KAY TAYLOR

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MELANIE KAY TAYLOR

Major Professor:	Nina Wurzburger
Committee:	Richard Lankau
	Robert Teskey

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
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DEDICATION

This thesis is dedicated to the memory of Ronald Ervin McNair, whose life and legacy has provided inspiration to many of us with humble beginnings.

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CHAPTER 1

INTRODUCTION

Mycorrhizal association types of plants determine patterns in soil biogeochemistry, from forest stands to biomes (Phillips et al. 2013, Averill et al. 2014). Ecosystems dominated by ectomycorrhizal (ECM) plants contain soils with an elevated ratio of carbon (C) to nitrogen (N) relative to those dominated by arbuscular mycorrhizal (AM) plants (Averill et al. 2014, Midgley and Phillips 2014), however it is unclear how these patterns have emerged. Proposed mechanisms include 1) distinct functional abilities of mycorrhizal and associated soil microbial communities and 2) differences in litter stoichiometry between mycorrhizal types, which may individually (or together) determine biogeochemical processes (Orwin et al. 2011, Phillips et al. 2013, Averill et al. 2014). Understanding these underlying mechanism(s) is critical in the context of the terrestrial C cycle and global change.

Nearly all plants participate in mycorrhizal symbioses, but mycorrhizal types are fundamentally different in partner identity, structure, and function (Smith and Read 2008). The most common mycorrhizal types are AM and ECM symbioses, which characterize most tree species in temperate forest ecosystems (Phillips et al. 2013). Arbuscular mycorrhizas are an ancient symbiosis, ancestral to all land plants, in which the fungal hyphae penetrate the plant root cells to form arbuscules, the organs that facilitate resource transfers between plant and fungus (Helgason and Fitter 2005). Arbuscular mycorrhizal fungi are obligate symbionts and generally aid in the acquisition of mineral nutrients, particularly N and phosphorus (P) (Smith and Read 2008, Smith and Smith 2011). In contrast, the ECM symbiosis is more recently evolved and

ECM fungi form a hyphal mantle around rootlets and an intracellular network between plant cells called the Hartig net, where transfers occur between plant and fungus (Smith and Read 2008). Unlike the AM symbiosis, the ECM symbiosis involves a diversity of fungal taxa that can acquire nutrients in mineral form and directly from organic matter (Churchland and Grayston 2014).

Arbuscular mycorrhizal and ECM fungi have distinct nutrient acquisition strategies. Arbuscular mycorrhizal roots and fungi rely on the diffusion of mineralized forms of nutrients towards roots and hyphae (Hodge and Fitter 2010, Nuccio et al. 2013), making them "scavengers" of soil nutrients. This trait is coupled with the tendency for AM plants to generate a bacterial-based energy channel (Bardgett and Wardle 2012), which promotes fast decomposition and the availability of inorganic nutrients. In contrast, ECM fungi not only acquire nutrients in mineral form, but can also acquire nutrients directly from soil organic matter (SOM) (Phillips et al. 2013). Ectomycorrhizal fungi accomplish this by excreting extracellular enzymes (Chalot and Brun 1998) to break down organic molecules and by taking up low-molecular weight organic molecules (Taylor et al. 2009), making them "miners" of soil nutrients. Ectomycorrhizal plants tend to promote a fungal-based energy channel (Bardgett and Wardle 2012), which may suppress decomposition and the availability of inorganic nutrients.

Mycorrhizal nutrient strategies mediate relationships with soil microbial communities and result in unique biogeochemical signatures on soils (McGuire et al. 2010). In the AM plant-soil system, saprotrophic organisms must mineralize nutrients from organic matter before they are readily available to AM fungi (Smith and Smith 2011). This suggests a coordinated functioning between saprotrophs and symbionts, where greater rates of SOM decomposition will ultimately yield greater nutrient availability for plants. In the ECM plant-soil system,

mycorrhizal mining of nutrients from SOM has two potential consequences. First, mining removes the reliance of mycorrhizal fungi on the mineralization pathway, and in a sense, creates a competitive context between symbionts and saprotrophs for organic nutrients (Averill et al. 2014). For example, ECM roots can suppress decomposition (Gadgil and Gadgil 1971) due to competition for soil nutrients and/or water between ECM fungi and decomposers (Koide and Wu 2003, Lindahl et al. 2010). Second, because ECM fungi acquire C from their host plant rather than from SOM, mining organic N may, over time, result in soils with an elevated C:N relative to AM soils (Orwin et al. 2011). The increase of SOM C:N may trigger a deficiency of N and promote differently structured decomposer communities in comparison to AM soils (Fierer et al. 2009).

The stoichiometry (i.e. C:N ratio) of leaf and root litter is another proposed mechanism for the difference in soil biogeochemistry between AM and ECM plants. Plant litter C:N can determine rates of litter decomposition (Gholz et al. 2000), which determines nutrient availabilities for plants and microbes. Additionally, varying litter C:N inputs over time can shape the structure of microbial communities (Strickland et al. 2009). Plant litter C:N and its effect on microbial community structure may contribute to the nature of C accumulation in SOM and, hence, soil C:N (Frank and Groffman 2009). Finzi et al. (1998b) observed that leaf litter C:N best explained variation in soil C:N in a temperate forest. Ectomycorrhizal tree species have leaves that tend to be associated with higher C:N (Vesterdal et al. 2008) and lower rates of decomposition relative to AM leaves in many ecosystems (Cornelissen et al. 2001, Vesterdal et al. 2012), but there is a large degree of variation among species and mycorrhizal types (Hobbie et al. 2006).

Root litter stoichiometry may also vary between AM and ECM plants. Root litter represents half of all litter inputs to soil in forests (Freschet et al. 2013), but is insufficiently represented in empirical studies compared to leaf litter. Root litter C:N is an important factor determining litter decomposition (Silver and Miya 2001), but few studies have reported the stoichiometry of root litter for AM and ECM plants (Hobbie et al. 2010). Mycorrhizal fungi have the ability to alter the decomposability of roots by changing root architecture and chemistry (Langley and Hungate 2003). While Urcleay et al. (2011) found that colonization by AM fungi did not alter the decomposability of various plant roots, Langley et al. (2006) demonstrated that pine roots colonized by ECM fungi decomposed more slowly than those that were uncolonized. This finding was attributed to the ECM fungal tissue, primarily made of chitin. However, recent studies have shown chitin to be relatively labile (Drigo et al. 2012, Fernandez and Koide 2012), and the initial melanin and N concentrations in ECM fungal tissue may be stronger regulators of ECM root decomposition (Fernandez and Koide 2014).

In this research, we sought to understand how mycorrhizal association types determine patterns in soil biogeochemistry among forest trees. Specifically, we tested two competing mechanisms for this phenomenon: 1) the distinct functional abilities of non-mycorrhizal microbial communities, and 2) leaf and root litter stoichiometry. To this end, our research was comprised of two parts: First, we characterized soil biogeochemical patterns (soil C:N, microbial biomass C:N and C:P, availabilities of ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-})) of individual trees of differing mycorrhizal association and litter stoichiometry in a well-mixed AM/ECM forest. We hypothesized that if mycorrhizal type is important in determining soil C:N, microbial biomass C:N, and available inorganic N, then there would be greater nutrient availability (N and P) and a lower microbial biomass C:N and C:P in AM versus ECM soils, but

that litter C:N would amend these effects. Second, we conducted an experiment on SOM decomposition to determine if functional attributes are different between mycorrhizal-specific microbial communities. We added leaf and root litter from each species to determine whether the response of microbial respiration depended on the mycorrhizal type of the tree from which soil was collected (soil mycorrhizal type) or the stoichiometry of litter. We hypothesized that heterotrophic soil respiration would be greater in AM soils vs. ECM soils and that respiration would decrease for both as soil C:N increased. In the context of litter addition, we hypothesized that the addition of low C:N litter would result in a similar respiration response in AM and ECM soils, but that the addition of high C:N litter would trigger greater respiration in ECM soils relative to AM soils. Specifically for the root litter additions, we hypothesized that increased mycorrhizal colonization would lead to decreased soil respiration response with ECM roots, but not AM roots.

CHAPTER 2

METHODS

Study Design and Site Description

In order to understand how tree species litter C:N and mycorrhizal association type determine patterns in soil biogeochemistry, we used two approaches with the same focal trees in a Georgia piedmont forest. First, in order to determine the soil and litter chemistry characterizing each target species, we measured total soil C and N, available N and P, pH, microbial biomass C, N, and P, and total litter C and N. Second, in order to determine if heterotrophic respiration is influenced by mycorrhizal association or soil C:N, we conducted a mesocosm experiment where we measured CO_2 efflux from soil from under AM and ECM tree species. Then, to determine the consequence of litter addition, we reciprocally crossed each species soil with each species litter.

We selected eight tree species for this experiment, including four AM species: tulip poplar (*Liriodendron tulipifera* L.), sweetgum (*Liquidambar styraciflua* L.), American holly (*Ilex opaca* Ait.), and Eastern red cedar (*Juniperus virginiana* L.) and four ECM species: mockernut hickory (*Carya tomentosa* Poir. Nutt.), American beech (*Fagus grandifolia* Ehrh.), white oak (*Quercus alba* L.), and loblolly pine (*Pinus taeda* L.). Each species is hereafter referred to by a species code consisting of the first two letters of the genus and specific epithet, respectively: LITU, LIST, ILOP, JUVI, CATO, FAGR, QUAL, and PITA.

All soil and litter samples were collected from Whitehall Forest, a mixed pine-hardwood research forest of the University of Georgia in Athens, Georgia, USA (33_ 570 N, 83_ 190 W, altitude 230 m) (Bauweraerts et al. 2014). The mature areas of hardwood forest at Whitehall

forest are approximately 70 years old (Danskin et al. 2009). All trees used for each species were mature, canopy-height specimens, with the exception of ILOP, which is a mid-story tree.

The climate of Whitehall Forest is humid subtropical (Dyer and Brook 1991), with a mean annual temperature of 16.59 °C and a mean annual precipitation of 105.25 mm (UGA-Libraries 2015). The land was cleared for agriculture by European settlers and used primarily for cotton production, which contributed to soil erosion. Agriculture was abandoned in the early 20th century and land has naturally reforested (Daniels 1987). Soils at Whitehall Forest are acidic Ultisols, classified as either Madison series sandy loam or Pacolet series sandy clay loam (Soil Survey Staff). The soil is highly eroded and characterized by low nutrient availability (Ford et al. 2005).

Soil Chemistry and Microbial Biomass

We collected soils from five trees of each species during the period of July-August 2014. All soil chemistry data from one CATO tree was removed from the dataset due to misidentification in the field. Individual trees from each species were sampled during five time points over a three-week period to ensure that temporal variability was represented equally across species. We targeted the top 10 cm of A horizon mineral soil. Twelve soil samples (10 cm deep, 2.5 cm diameter), were collected from each tree: six samples were taken from random locations within 20 cm of the main stem and six were taken from random locations approximately one m away from the main stem. We homogenized all soil samples from an individual tree and roots, rocks, and debris were discarded. Extractions of soil nitrate (NO_3^-), ammonium (NH_4^+), and phosphate (PO_4^-) were initiated upon sampling in the field (detailed below). In the lab, soils were sieved (2 mm) and microbial biomass extractions were initiated (1-3 hours after collection; detailed below). Gravimetric soil moisture was determined by drying

samples of each soil at 60° C for 72 hours. Dried soils were ground to a fine powder and analyzed for total C and N using a NA1500 C/H/N Analyzer (Carlo Erba Strumentazione, Milan, Italy). To determine soil pH, 10 g of the oven-dried equivalent of fresh soil from the pooled soil for each species was mixed with 10 mL DI H_2O to form a soil slurry. Four slurries were created for each species and placed on a shaker (150 rpm) for 30 minutes. Soil pH was measured using a calibrated Accumet basic AB15 electrode (Fisher Scientific, Massachusetts, USA).

To determine pools of inorganic N, we extracted soil with potassium chloride (KCl) by combining approximately 40 g of soil with 50 mL 2 M KCl, placed it on a shaker (150 rpm) for 3-4 hours, filtered through paper (Whatman 41, GE Biosciences, Pennsylvania, USA) and then through a microbial syringe filter (Acrodisc, 1 μm glass fiber membrane, Pall Life Sciences, Ann Arbor, MI). Samples were frozen at -20°C until analysis and analyzed for NO_3^- and NH_4^+ using colorimetry on a continuous flow autoanalyzer (Alpkem 301, Oregon, USA). Values are reported as ng NO_3^- per g soil (ppb) or μg NH_4^+ per g soil (ppm). Reported DIN values are the sum of NO_3^- and NH_4^+ and are expressed in μg per g soil (ppm).

To determine pools of PO_4^{3-}, approximately 12 g of soil from each sample was combined with a charged 3 x 7 cm anion resin strip (AMI-7001, Membranes International, Ringwood, NJ) and 40 mL DI H_2O and placed on a shaker (150 rpm) for 24 hours. Resin strips were later rinsed with DI H_2O, eluted with 40 mL 2 M KCl, and placed on a shaker at 150 rpm for 1 hour. The extractant was then filtered through paper (Whatman 41, GE Biosciences, Pennsylvania, USA) and then through a microbial syringe filter (Acrodisc, 1 μm glass fiber membrane, Pall Life Sciences, Ann Arbor, MI). Samples were frozen at -20 °C until analysis and analyzed for PO_4^{3-} using colorimetry on a 301 continuous flow autoanalyzer (Alpkem 301, Oregon, USA). Values are reported as μg PO_4^{3-} per g soil (ppm).

To determine microbial biomass C,N, and P, two 7 g subsamples of each soil were mixed with either 40 mL 0.5 M potassium sulfate (K_2SO_4) (unfumigated) or a mixture of 40 mL 0.5 M K_2SO_4 and 0.5 mL ethanol-free chloroform ($CHCl_3$) (fumigated) and placed on a shaker for three hours at 150 rpm. Fumigated samples were bubbled vigorously using a fumigation manifold for 30 minutes to remove $CHCl_3$ and all samples were then filtered through paper (Whatman 42, GE Biosciences, Pennsylvania, USA) and then through a microbial syringe filter (Acrodisc, 1 μm glass fiber membrane, Pall Life Sciences, Ann Arbor, MI). Samples were frozen at -20°C until analysis. Total dissolved C (TDC) was measured using a TOC analyzer (Shimadzu, TOC-5000A, Kyoto, Japan). Total dissolved N (TDN) was measured by processing extracts through a persulfate digestion (Cabrera and Beare 1993) and then analyzing them using colorimetry on a 301 continuous flow autoanalyzer (Alpkem 301, Oregon, USA). Total dissolved P (TDP) was measured using colorimetry on a 301 continuous flow autoanalyzer (Alpkem 301, Oregon, USA). Microbial biomass C, N, and P values were calculated by taking the difference between unfumigated and fumigated samples and are reported as μg TDC per g soil, μg TDN per g soil, and μg TDP per g soil. Microbial biomass C:N is the ratio of TDC and TDN values. Microbial biomass C:P is the ratio of TDC and TDP values.

Soil and soil x litter mesocosm experiment

Soil was collected from the same individual trees using the 12-probe method described above in January 2014. The top 10 cm of A horizon mineral soil was targeted. Soil samples were homogenized and taken back to the laboratory, sieved (2 mm), and then stored at 5°C for 48-72 hours before being introduced to mesocosms. Subsamples were dried using the same procedure described above to determine soil moisture and to calculate target soil weights (see below).

Leaf and root litter were collected from 5-7 individuals of each species at Whitehall Forest from September-December 2013 using litter traps for catching senesced leaves and tracing roots from the base of target trees for root material. Minimal error is expected due to the use of living roots since litter chemistry has been shown to be similar between living and dead roots (Gordon and Jackson 2000). Roots were traced out from the main stem in order to ensure species identity. Roots greater than 2 mm in diameter were discarded and the remaining roots were cleaned with paintbrushes in DI H_2O, surface sterilized in a 30% hydrogen peroxide (H_2O_2) solution for 1 minute, then triple rinsed in DI H_2O. A subsample of fresh root tissue was preserved in a 70% ethanol solution for subsequent mycorrhizal analysis. Leaves and roots were dried at 40°C for 48 hours and fragmented to approximately 1 cm pieces with scissors before allocation into experimental mesocosms. Representative subsamples of all soil and litter samples were ground to a fine powder and analyzed for total C and N using a NA1500 C/H/N Analyzer (Carlo Erba Strumentazione, Milan, Italy).

Homogenized soil samples from each tree species (10 g dry weight equivalent) were placed in 50 cm^3 centrifuge tubes, which then received one of eight leaf litter treatments, one of eight root litter treatments or served as a soil-only control. Soils of each species were crossed with leaf litter from each species in a fully factorial design, (with the same design for root litter) with three replicates of each combination to create 192 leaf-soil mesocosms, 192 root-soil, and 24 soil-only mesocosms for a total of 408 mesocosms. Mesocosms were covered with perforated film, maintained at 25°C, and monitored weekly to maintain moisture content with additions of DI H_2O.

Mesocosms were sampled for respiration five times over the 140 day experiment. During a sampling event, individual mesocosms were flushed with pure N_2 to reduce background carbon

dioxide (CO_2) and capped with a gas tight lid outfitted with a septum. A needle attached to a 3 cm^3 syringe was inserted through the septum, the syringe was plunged vigorously six times to mix the headspace before collecting a 2 cm^3 sample. Gas samples were analyzed for CO_2 concentration using an infrared-gas analyzer (LiCor 6252, LiCor, Lincoln, NE) and a standard curve generated from a gas standard (Airgas, 1000 ppm CO_2, Radnor, PA). During each sampling event, gases were sampled at three consecutive time points over an hour and half incubation to determine the rate of respiration, or CO_2 efflux (μmol CO_2/g C/hr) for a given time point. Total respiration, or cumulative CO_2 loss (over the 140 day experiment) was estimated by extrapolating these measured rates between the sampling points. Cumulative CO_2 loss is expressed as μmol CO_2/g C (in both soil and litter) over the 140 days of the experiment.

To determine the effect of litter addition on respiration, an additional parameter was calculated, CO_2 deviation, in which the mean cumulative soil respiration (CO_2 efflux) for soil from under each tree species was subtracted from each litter addition mesocosm with soil from under that particular tree species. This parameter was calculated in order to isolate the effects of the litter addition treatment from the pre-existing soil related effects.

Mycorrhizal colonization

Mycorrhizal colonization was quantified from root samples (pooled samples from five individual trees of each species) that had been preserved in 70% ethanol and stored at 5°C. For ECM trees, roots were rinsed in DI H_2O and colonization was quantified in two ways: First, we used the "grid method" to determine the percentage of root length with mycorrhizal colonization (a fungal mantle). Second, we used the "tip method" by quantifying the percentage of roots tips with mycorrhizal colonization. For AM trees, we rinsed the preserved roots in DI H_2O, and then cut each root into small (2 cm) fragments. In accordance with the method used by Cornelissen

(2003), roots were placed in a 5% KOH solution and then on a 60°C water bath for 5-7 hours, with the KOH solution being replaced every two hours. The roots were then triple rinsed with DI H_2O and soaked in a 2% HCl solution for 3 minutes to acidify the roots. Next, the roots were placed in a 0.05% trypan blue solution for staining (trypan blue in 2:1:1 lactic acid, water, glycerol) in the 60°C water bath for 15 minutes. The roots were rinsed and stored in a destain solution (1:1:1 lactic acid, water, glycerol) until microscopic analysis. Roots were analyzed for mycorrhizal structures using a modified version of the method described by McGonigle (1990) in which we mounted 10 sections of root from each pooled species sample onto a microscope slide. We then used six intercepts on each root section to quantify presence or absence of arbuscules, vesicles, and hyphae at 40x magnification. The data presented are the total percentages of all intercepts containing arbuscules, vesicles, and hyphae, respectively.

Statistical Analyses

To examine the mycorrhizal and litter stoichiometry effects on biogeochemical patterns in soil and heterotrophic respiration, we used linear mixed-effect models in R (version 3.1.1, package: lme4) (Bates et al. 2014). For the biogeochemical analyses involving N (soil C:N, microbial biomass C:N, NH_4^+, NO_3^-, DIN), mycorrhizal type, leaf C:N, and root C:N were treated as fixed effects and species was treated as a random effect. If there was no significant interactive effect ($p>0.05$) of soil mycorrhizal type and litter C:N (either leaf or root), a new model was created in which they were treated as separate terms. Specifically for the analyses of soil C:N involving averaged leaf and root litter C:N (Average C:N), mycorrhizal type and average C:N were treated as fixed effects while species was treated as a random effect. For microbial biomass C:N, soil C:N was added as an additional fixed effect. For the biogeochemical analyses not involving N (PO_4^{3-}, microbial biomass C:P), litter C:N was excluded from the

model terms such that mycorrhizal type was the sole fixed effect and species was the random effect. Reported p-values were obtained using the likelihood ratio test. Model constructions are reported in supplemental table 1.

Litter C:N was measured with replicates from pooled litter samples such that there was one mean value for each litter species. We used Welch's t-test to test the difference between AM and ECM leaf and root litter C:N.

Since soil pH was measured using technical replicates from pooled species samples, and therefore one soil pH mean value was available for each species, we created a linear model with soil mycorrhizal type, leaf litter C:N, and root litter C:N as main effects. The terms were allowed to interact and when no interaction was found, the terms were treated separately. Reported p-values were obtained from analysis of variance (ANOVA).

To examine heterotrophic respiration from soil-only mesocosms, mycorrhizal type and soil C:N were treated as fixed effects and soil species was treated as a random effect. For the litter addition portion of the study, both the effects of leaf and root litter were first considered together. We constructed a linear mixed-effects model where litter type and litter C:N were the fixed effects and soil species and litter species were the random effects. After this analysis, leaf and root litter additions were treated separately to determine the mycorrhizal effect. CO_2 deviation was treated as the response variable and soil mycorrhizal type, litter mycorrhizal type, and litter C:N (either root or leaf) were treated as fixed effects with soil species and litter species as non-interacting random effects. Soil mycorrhizal type and litter C:N were initially set up as an interaction and if none was significant ($p>0.05$), a second model was proposed where each fixed effect was a separate term. Reported p-values were obtained using the likelihood ratio test.

To determine if mycorrhizal colonization was important for determining CO_2 deviation, we created separate mixed effects models for AM and ECM root litters. These models used mycorrhizal colonization as the sole fixed effect and soil species and litter species as random effects. For ECM roots, the grid and tip methods were analyzed in separate models. For AM roots, arbuscules, vesicles, and hyphae were analyzed in separate models. Reported p-values were obtained using the likelihood ratio test.

CHAPTER 3

RESULTS

Litter and Soil Chemistry

Litter C:N was not significantly different between AM and ECM trees (Table 2). Ectomycorrhizal tree leaf litter tended to have a higher C:N ($t_{(5.54)}$= -1.77, p=0.13) than AM tree leaf litter, though root litter C:N was not different between mycorrhizal types ($t_{(3.24)}$= -0.51, p=0.64).

Soil chemistry was largely determined by either mycorrhizal type or an interaction of mycorrhizal type and litter C:N. In the mixed-effects model used to analyze soil C:N, soil C:N was determined by the interaction of mycorrhizal type and leaf litter C:N ($\chi^2_{(1)}$=4.21, p=0.04) (Figure 1) and the interaction of mycorrhizal type and root litter C:N ($\chi^2_{(1)}$=5.38, p=0.02) (Figure 2). Soil C:N from AM trees decreased slightly with increasing leaf litter C:N, while soil C:N from ECM trees increased sharply with increasing leaf litter C:N. The interaction of mycorrhizal type and root litter C:N is questionable due to the narrow range of ECM root litters represented (see discussion). When mycorrhizal type and root litter C:N are treated as non-interacting terms, soil C:N is not well explained by root litter C:N ($\chi^2_{(1)}$=0.24, p=0.63). When leaf and root litter C:N was averaged, there was no significant interaction of mycorrhizal type and average C:N ($\chi^2_{(1)}$=1.79, p=0.18). When the terms were separated, average C:N was close to significant for explaining soil C:N ($\chi^2_{(1)}$=2.88, p=0.09), but mycorrhizal type was not ($\chi^2_{(1)}$=0.57, p=0.45). All mixed-model constructions and effect estimates are summarized in supplemental table 1.

Total dissolved inorganic N was best explained by the interaction of mycorrhizal type and root litter C:N ($\chi^2_{(1)}$=4.11, p=0.04) and leaf litter C:N had no effect ($\chi^2_{(1)}$=0.74, p=0.39). When root litter C:N and mycorrhizal type were treated separately, root litter C:N was close to significant ($\chi^2_{(1)}$=2.88, p=0.09). Ammonium availability followed the same pattern as total DIN. Ammonium was explained by the interaction of mycorrhizal type and root litter C:N ($\chi^2_{(1)}$=3.60, p=0.06) (Figure 3). Leaf litter C:N had no effect on ammonium availability ($\chi^2_{(1)}$=0.63, p=0.43). When root litter C:N and mycorrhizal type were treated separately, root litter C:N was close to significant ($\chi^2_{(1)}$=2.89, p=0.09), but mycorrhizal type was not ($\chi^2_{(1)}$=0.05, p=0.83). For the nitrate analysis, one value was excluded as an outlier due to being one order of magnitude above the average recorded value (species: CATO). Nitrate availability was not affected by mycorrhizal type ($\chi^2_{(1)}$=1.00, p= 0.32), leaf litter C:N ($\chi^2_{(1)}$=0.03, p= 0.86) or root litter C:N ($\chi^2_{(1)}$= 0.45, p=0.50) (Table 1; Figure 4).

In the linear model used to analyze soil pH, soil pH was unaffected by tree mycorrhizal type ($F_{(1,4)}$= 2.23, p=0.21) and leaf litter C:N ($F_{(1,4)}$= 0.66, p=0.46) and root litter C:N ($F_{(1,4)}$= 0.05, p=0.84) (Table 2). For phosphate, two data points were excluded as outliers due to being one order of magnitude over the average recorded value (species: JUVI and LITU). Phosphate availability was not affected by mycorrhizal type ($\chi^2_{(1)}$=0.29, p=0.59) (Table 1; Figure 5).

Microbial Biomass

ECM soils had a higher microbial biomass C:N ($\chi^2_{(1)}$=4.14, p=0.04) than AM soils (Figure 6) and microbial biomass C:N was positively correlated with soil C:N ($\chi^2_{(1)}$=3.29, p=0.07) (Figure 7). Microbial biomass C:N was not affected by either leaf litter C:N ($\chi^2_{(1)}$=0.01, p=0.55) or root litter C:N ($\chi^2_{(1)}$=0.06, p=0.81) (Table 1). Two values were excluded from the microbial biomass C analysis as outliers due to being greater than two standard deviations away

from the mean (species: JUVI). These values were also excluded from the microbial biomass C:N and C:P data. Microbial biomass C:P was unaffected by mycorrhizal type ($\chi^2_{(1)}$=0.91, p=0.34) (Table 1; Figure 8). For microbial biomass P, four samples were not analyzed due to insufficient extraction volumes (species: FAGR, ILOP, LITU, QUAL).

Soil and soil x litter mesocosm experiment

With soil-only treatments, total CO_2 efflux over the 140 day experiment was higher in AM soils than ECM soils (Figure 9) and there was a strong interaction of soil mycorrhizal type and soil C:N ($\chi^2_{(1)}$=6.82, p<0.01). Total respiration in AM soils increased with increasing soil C:N while respiration in ECM soils was insensitive to soil C:N (Figure 10).

Across all litter addition treatments, CO_2 deviation was determined by litter type (leaf/root) ($\chi^2_{(1)}$=136.33, p<0.001) and litter C:N ($\chi^2_{(1)}$=20.70, p<0.001). CO_2 deviation was higher amongst leaf litter additions than root litter additions, even across a range of similar litter C:N. Increasing litter C:N was associated with a decrease in CO_2 deviation across all litter additions (Figure 11).

In leaf-litter addition treatments, there were no interactive effects of soil mycorrhizal type and litter C:N on CO_2 deviation, so each term was treated separately (Supplemental Table 1). CO_2 deviation was not well explained by soil mycorrhizal type ($\chi^2_{(1)}$=0.03, p=0.86), litter mycorrhizal type ($\chi^2_{(1)}$=0.48, p=0.49), or leaf litter C:N ($\chi^2_{(1)}$=0.70, p=0.40; Figures 12 and 13).

In root-litter addition treatments, there were no interactive effects of soil mycorrhizal type and litter C:N on CO_2 deviation, so each term was treated separately. CO_2 deviation was affected by soil mycorrhizal type ($\chi^2_{(1)}$=2.81, p= 0.09), litter mycorrhizal type ($\chi^2_{(1)}$=7.23, p<0.01), and litter C:N ($\chi^2_{(1)}$=6.38, p=0.01) (Figures 14 and 15).

Arbuscular and ECM root colonization were analyzed separately with mixed-effects models (Supplemental table 1; Table 3). For ECM roots, neither the grid method nor the tip method of quantifying colonization yielded results that affected CO_2 deviation ($\chi^2_{(1)}$=0.02, p=0.88; $\chi^2_{(1)}$=0.003, p=0.96, respectively). For AM roots, CO_2 deviation was not affected by the percentage of arbuscules ($\chi^2_{(1)}$=1.90, p=0.17), vesicles ($\chi^2_{(1)}$=0.81, p=0.37), or hyphae ($\chi^2_{(1)}$=0.28, p=0.60).

Table 1: Soil chemical characteristics from each tree species (n=5 trees for each species) and mycorrhizal type (AM and ECM). Soil C:N values are (% total C/% total N). Nitrate values are in ng/g soil (ppb). Ammonium and phosphate values are in μg/g soil (ppm). Microbial biomass (MB) C:N is the elemental ratio (μg total dissolved C per g soil/ μg total dissolved N per g soil). Microbial biomass C:P is the elemental ratio (μg total dissolved C per g soil/ μg total dissolved P per g soil). Values represent mean and standard error (SE) for each species and mycorrhizal type.

Species	**Soil C:N**	**NO_3^- (ppb)**	**NH_4^+ (ppm)**	**PO_4^{3-} (ppm)**	**MB C:N**	**MB C:P**
AM	**19.66 (0.83)**	**27.57 (8.15)**	**1.10 (0.22)**	**0.26 (0.05)**	**10.42 (0.69)**	**281.42 (80.80)**
LITU	18.55 (2.09)	42.44 (23.07)	1.42 (0.55)	0.32 (0.16)	9.48 (0.51)	212.37 (35.63)
LIST	19.73 (1.80)	22.08 (10.38)	0.85 (0.16)	0.22 (0.09)	12.11(1.23)	216.97 (61.37)
ILOP	21.00 (2.01)	28.48 (22.34)	0.52 (0.12)	0.18 (0.09)	9.57 (1.98)	513.41 (313.20)
JUVI	19.37 (0.74)	17.27 (5.88)	1.61 (0.60)	0.22 (0.03)	10.57 (1.17)	171.59 (36.24)
ECM	**20.14 (1.06)**	**13.25 (3.64)**	**0.86 (0.16)**	**0.23 (0.04)**	**12.78 (0.65)**	**609.19 (260.40)**
CATO	15.26 (0.36)	17.09 (10.50)	1.22(0.35)	0.35 (0.10)	12.68 (2.39)	231.87 (18.78)
FAGR	18.51 (1.64)	14.03 (6.57)	0.59(0.08)	0.21 (0.07)	13.08 (1.60)	161.88 (40.07)
QUAL	21.77 (0.78)	14.23 (9.15)	1.06(0.18)	0.18 (0.05)	11.98 (0.67)	1801.91 (990.70)
PITA	24.02 (2.47)	9.18 (6.01)	1.42(0.53)	0.20 (0.05)	13.36 (0.75)	314.72 (21.14)

Table 2: Soil pH and leaf and root litter C:N for each species and mycorrhizal type (AM and ECM). Leaf and root C:N represent the ratio of total % C to total % N of leaf and root material. Values represent mean and standard error (SE) for each species and mycorrhizal type.

	Species	Soil pH	Leaf C:N	Root C:N
AM		**6.05 (0.18)**	**43.57 (4.26)**	**57.64 (10.82)**
	LITU	6.10 (0.02)	47.92 (0.45)	32.33 (0.34)
	LIST	6.52 (0.05)	53.41 (0.52)	59.82 (0.31)
	ILOP	5.84 (0.02)	35.55 (0.55)	84.91 (0.70)
	JUVI	5.73 (0.01)	37.40 (0.54)	53.51 (0.41)
ECM		**5.63 (0.17)**	**56.21 (5.73)**	**63.26 (2.16)**
	CATO	6.02 (0.03)	49.96 (1.06)	68.47 (0.41)
	FAGR	5.33 (0.06)	43.55 (0.67)	58.40 (0.28)
	QUAL	5.83 (0.03)	62.94 (0.65)	64.69 (1.57)
	PITA	5.36 (0.05)	68.41 (1.01)	61.48 (2.38)

Table 3: Mycorrhizal colonization from pooled root samples from each species and mycorrhizal type (AM and ECM). Mycorrhizal colonization for AM species is expressed as the percentage of root length with arbuscules, vesicles, and hyphae. Mycorrhizal colonization for ECM species is expressed as a percentage of root length (grid method) or a percentage of tips colonized by an ECM mantle. Values represent mean and standard error (SE) for each species and mycorrhizal type.

	Species	**Mycorrhizal colonization**		
		% Arbuscules	**% Vesicles**	**% Hyphae**
AM		**37.50 (0.08)**	**65.63 (0.17)**	**84.90 (0.11)**
	LITU	47.92 (0.10)	77.08 (0.06)	85.42 (0.07)
	LIST	45.83 (0.04)	83.33 (0.05)	100 (0)
	ILOP	12.50 (0.05)	14.58 (0.06)	54.17 (0.06)
	JUVI	43.75 (0.08)	87.50 (0.07)	100 (0)
		% Root length	**% Tip**	
ECM		**30.67 (0.03)**	**59.81 (0.12)**	
	CATO	23.25 (0.06)	25.04 (0.02)	
	FAGR	33.62 (0.06)	72.51 (0.08)	
	QUAL	31.81 (0.02)	63.85 (0.06)	
	PITA	34.01 (0.002)	77.86 (0.02)	

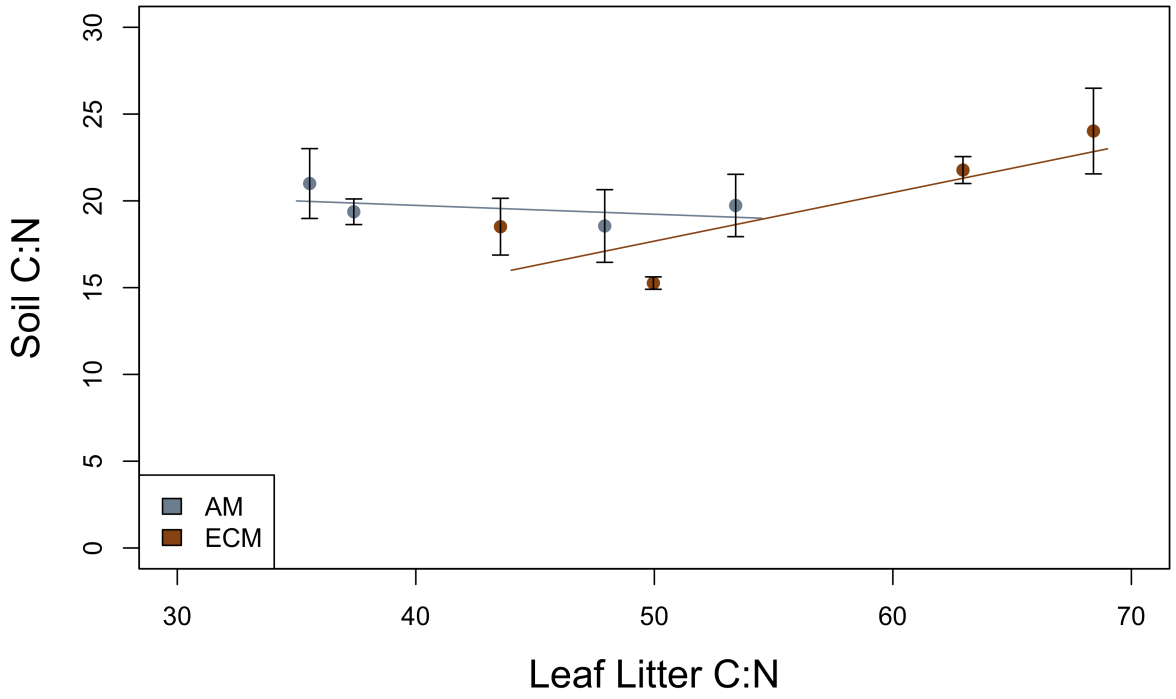

Figure 1: The relationship between soil C:N and leaf litter C:N across eight tree species. A significant interaction exists for mycorrhizal type and leaf litter C:N and mycorrhizal type and root litter C:N (Figure 2). The slopes for the linear models used to create these best-fit lines are not significantly different from zero, but the slopes are significantly different from each other. Points represent mean leaf and soil C:N for each species and error bars represent +/- standard error (SE) of soil C:N.

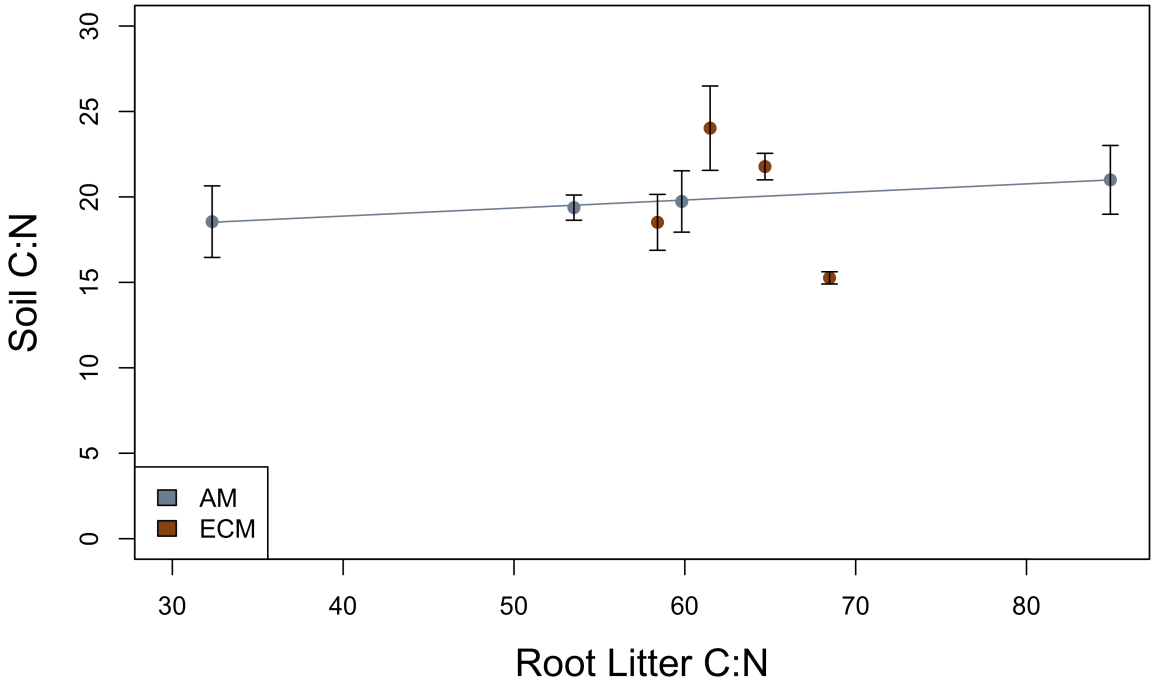

Figure 2: The relationship between soil C:N and root litter C:N across eight tree species. Soil C:N is determined by the interaction of mycorrhizal type and leaf litter C:N (Figure 1) and the interaction of mycorrhizal type and root litter C:N. Due to the limited range of ECM root litter C:N, we have omitted the ECM best fit line. The slope of the best-fit line for AM points is significantly different from zero, but the linear function for ECM points is not. Points represent mean root and soil C:N for each species and error bars represent +/- standard error (SE) of soil C:N.

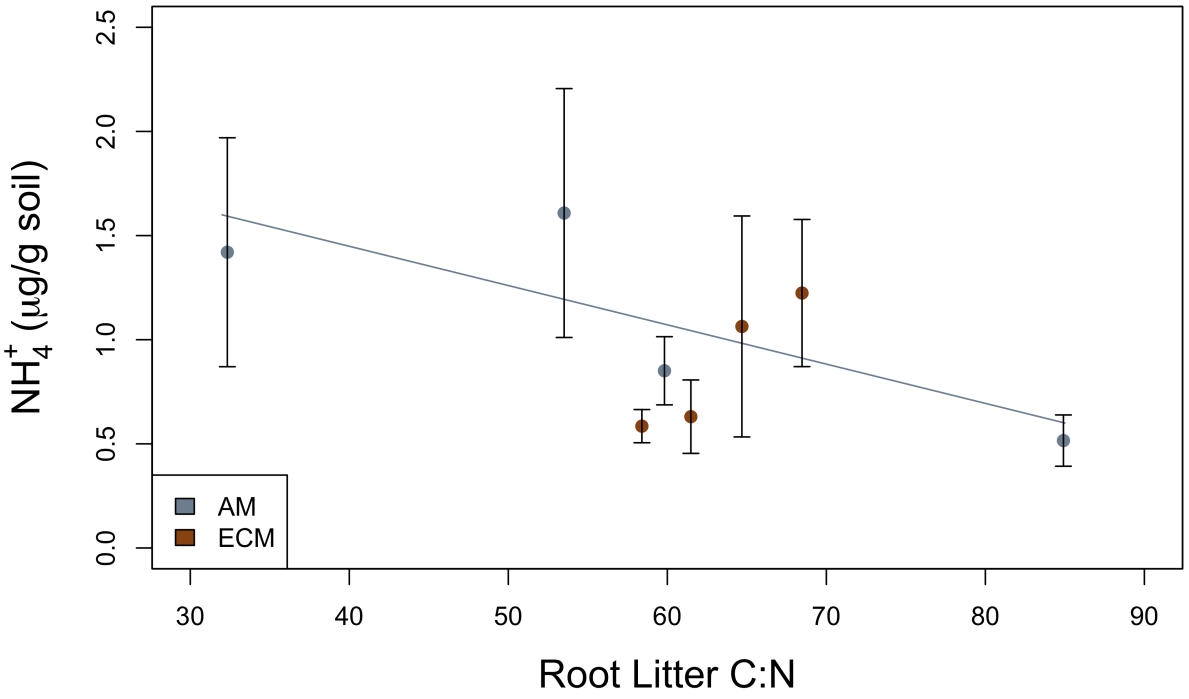

Figure 3: The relationship between ammonium (NH_4^+) availability and root litter C:N of AM and ECM trees. There is a significant interaction of mycorrhizal type and root litter C:N, but this may be driven by the narrow range of ECM root litter C:N. Therefore, we have omitted a best fit line through the ECM values. The slope of the AM best-fit line is not significantly different from zero. Values represent means and standard error (SE).

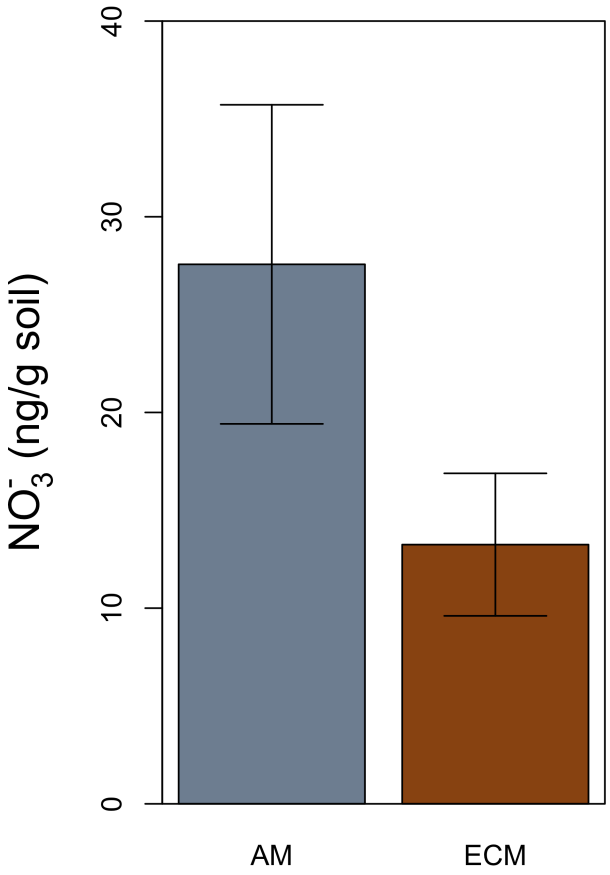

Figure 4: Nitrate (NO_3^-) availability of AM and ECM tree soils. The difference between AM and ECM was not statistically significant and litter stoichiometry did not affect nitrate availability. Values are means and standard error (SE).

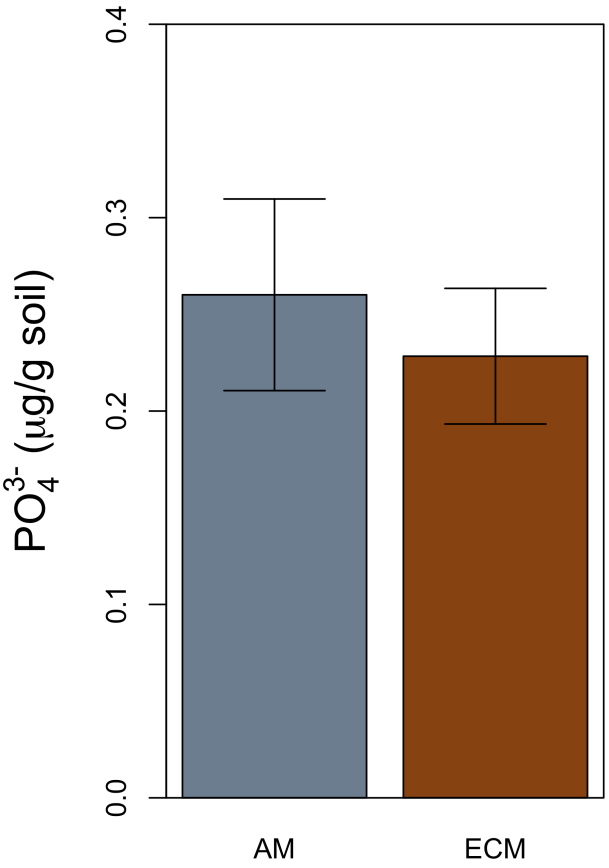

Figure 5: Phosphate (PO_4^{3-}) availability of AM and ECM tree soils. Phosphate is not significantly different between mycorrhizal types and was unaffected by litter C:N. Values represent means and standard error (SE).

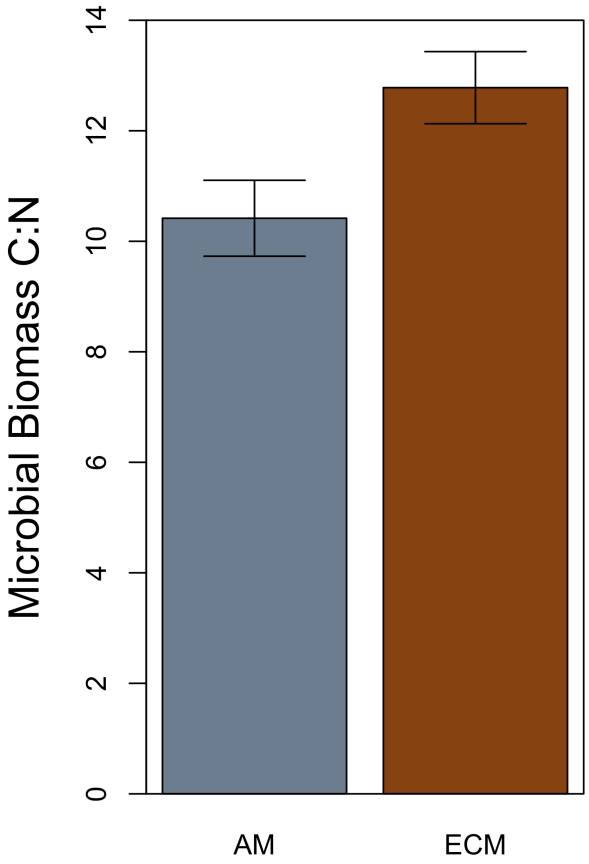

Figure 6: Microbial biomass C:N from AM and ECM tree soils. Values are elemental ratios (μg total dissolved C per g soil/ μg total dissolved N per g soil). ECM soils have a higher microbial biomass C:N than AM soils, but litter C:N did not affect microbial biomass C:N. Values represent means and standard error (SE).

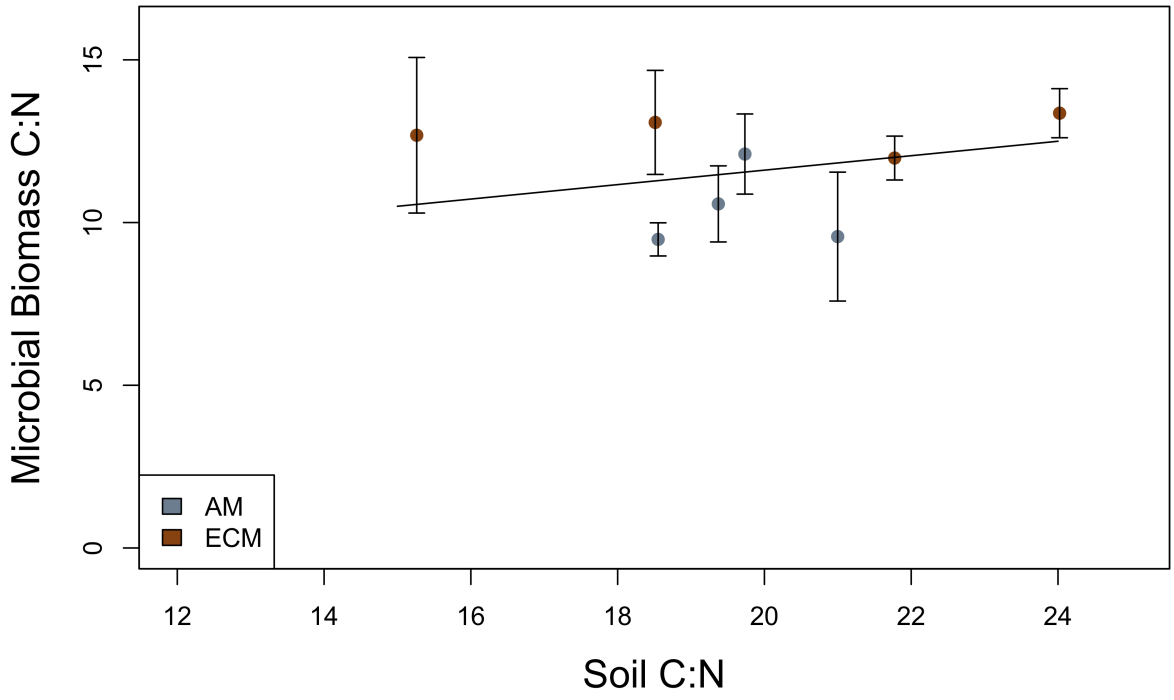

Figure 7: The relationship of microbial biomass C:N and soil C:N. Microbial biomass C:N values are elemental ratios (µg total dissolved C per g soil/ µg total dissolved N per g soil). Soil C:N is the ratio of total C per g soil to total N per g soil. Microbial biomass C:N is significantly higher in ECM soils compared to AM soils. Values represent means and standard error (SE).

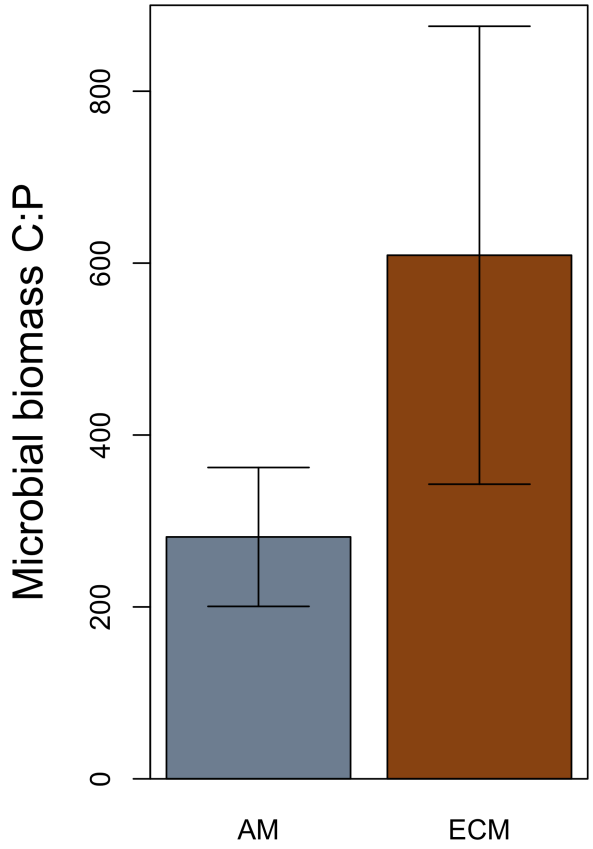

Figure 8: Microbial biomass C:P values of AM and ECM tree soils. Values are elemental ratios (μg total dissolved C per g soil/ μg total dissolved P per g soil). Microbial biomass C:P values did not differ significantly between mycorrhizal types or across litter C:N. Values represent means and standard error (SE).

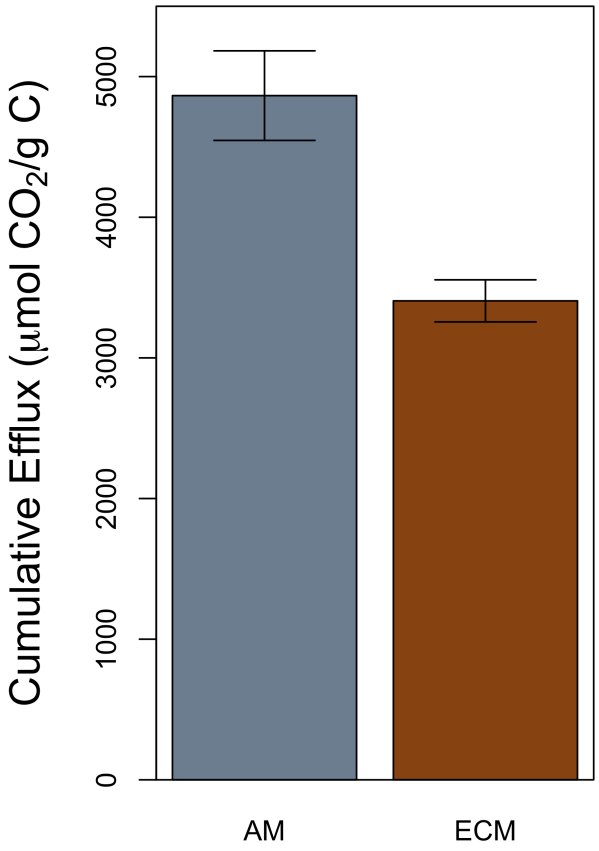

Figure 9: Total soil respiration (cumulative CO_2 efflux) from AM and ECM soils for the 140 days of the experiment. AM soils respired significantly more than ECM soils. Values represent means and standard error (SE).

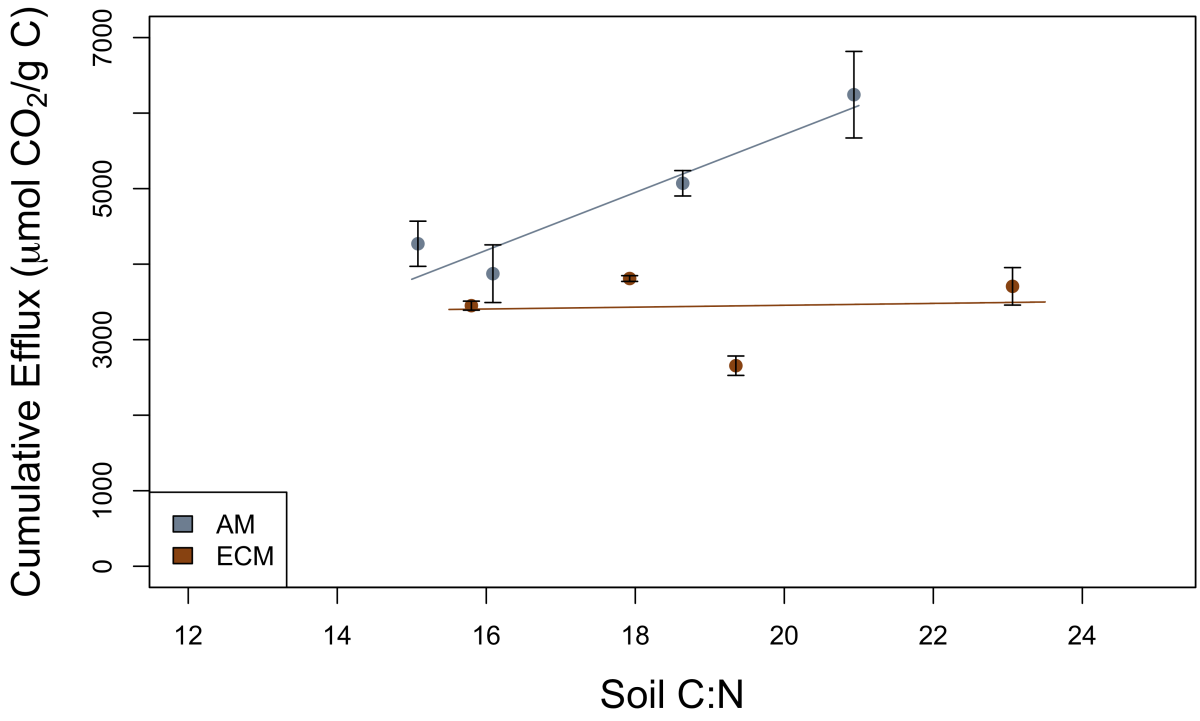

Figure 10: Total soil respiration (cumulative CO_2 efflux) in soils with no litter additions relative to soil C:N for each species. There is a significant interaction of mycorrhizal type and soil C:N. ECM soils respired similar amounts of CO_2 across a range of soil C:N, but AM soils respire increasing amounts of CO_2 as soil C:N increases. Values represent means and standard error (SE).

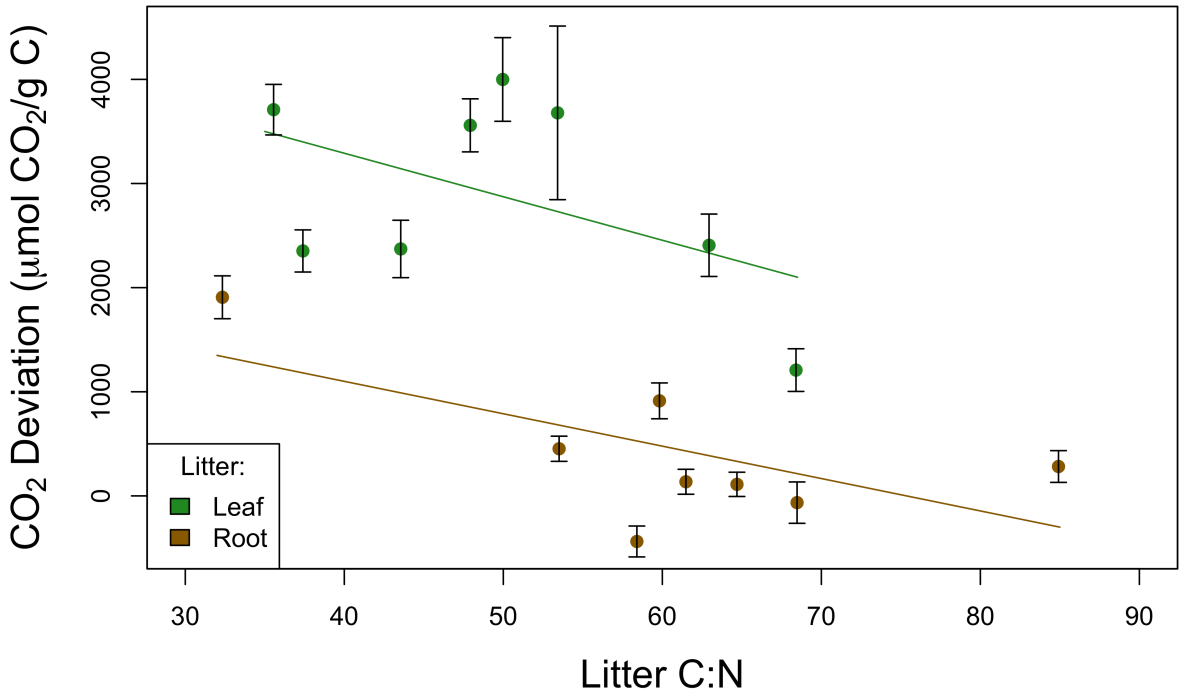

Figure 11: The response of soil respiration to litter addition (CO_2 deviation) and its relationship to litter C:N of leaves and roots averaged across all soil species. Leaf litter addition enhanced respiration relative to that of roots. Litter C:N was significantly correlated with a decline in respiration for both litter types, but not individually for leaf and root litter. Negative values indicate a suppression of respiration in comparison to the soil-only treatments. Values represent means and standard error (SE).

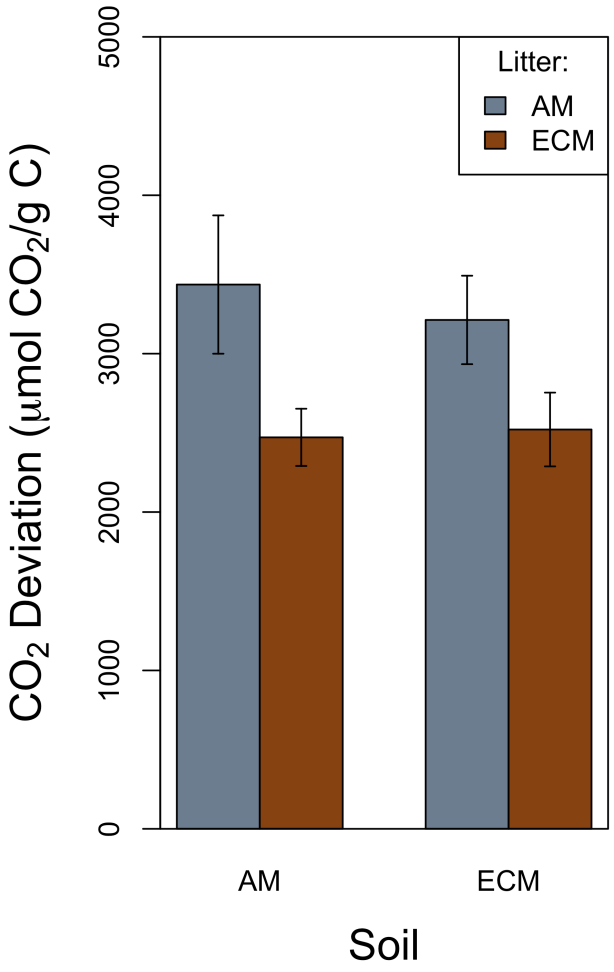

Figure 12: The response of soil respiration to leaf litter addition (CO_2 deviation) in AM and ECM soils with AM and ECM leaf litter. Additions of ECM leaf litter dampened the response of respiration in both AM and ECM soils compared to that of AM leaf litter. Values represent means and standard error (SE).

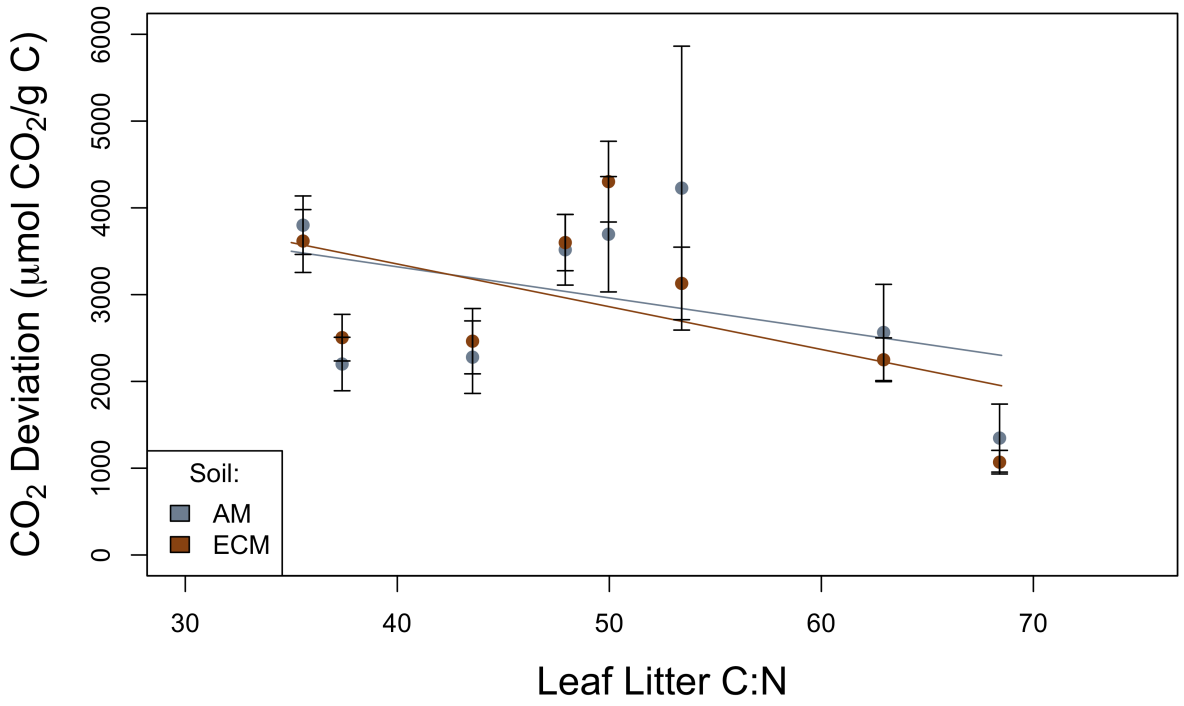

Figure 13: The response of soil respiration to leaf litter addition (CO_2 deviation) across leaf litter C:N for AM and ECM soils. Values represent means and standard error (SE).

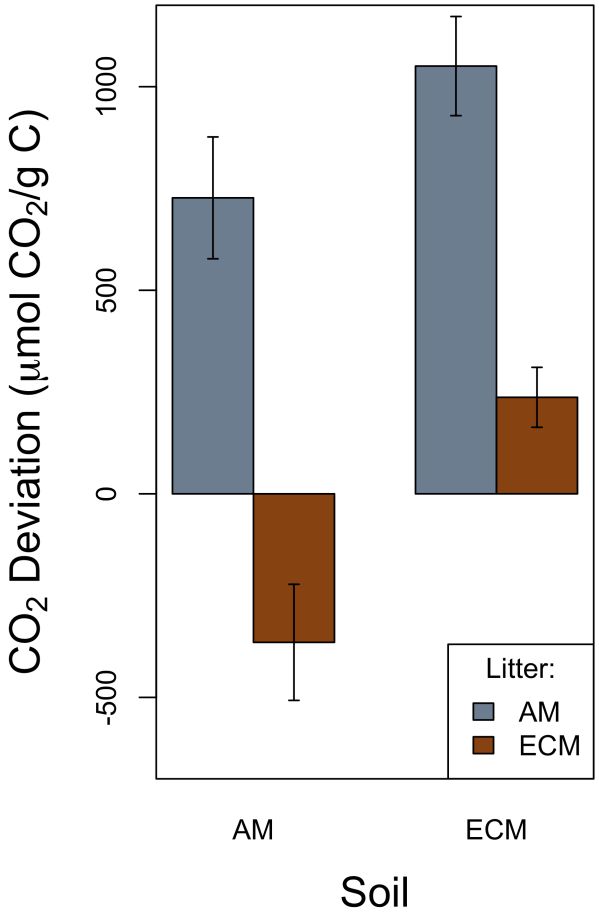

Figure 14: The response of soil respiration to root litter addition (CO_2 deviation) from AM and ECM soils with AM and ECM litters. The respiration response to litter addition was greater in ECM soils relative to AM soils and greater for AM versus ECM root litter. ECM root litter suppresses respiration in AM soils. Values represent means and standard error (SE).

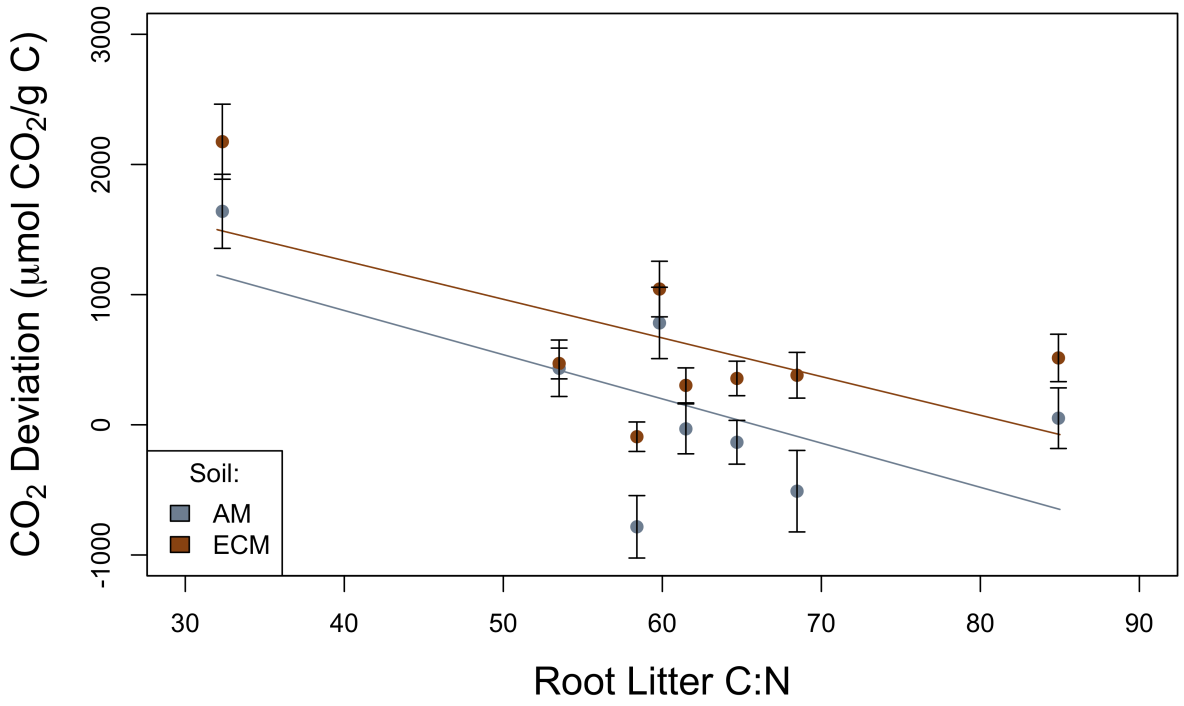

Figure 15: The response of soil respiration to root litter addition (CO_2 deviation) relative to root litter C:N in AM and ECM soils. The respiration response to litter addition declines with increasing root litter C:N. Values represent means and standard error (SE).

CHAPTER 4

DISCUSSION

Our results demonstrate that soil C and N cycling differs between AM and ECM tree species in a mixed-mycorrhizal temperate forest. This finding supports biome-wide patterns of soil C:N (Averill et al. 2014, Midgley and Phillips 2014) and confirms that differences in mycorrhizal types can manifest at the level of individual forest trees (Finzi et al. 1998a, Finzi et al. 1998b). Our study produced new findings, determining that both litter stoichiometry and the functional abilities of heterotrophic communities contribute to the differences in soil biogeochemistry between mycorrhizal types.

In our study, soil C:N was not distinctly different between AM and ECM tree species, as was also demonstrated in previous studies (Finzi et al. 1998b, Vesterdal et al. 2008). Instead, and in accordance with our expectation, soil C:N was best explained by an interaction of mycorrhizal type and leaf litter C:N. Soil C:N was relatively consistent among AM trees, while soil C:N from ECM trees increased with increasing leaf litter C:N (Figure 1). This pattern suggests that C and N in litter are processed together by the AM soil microbial community, leading to a microbial C:N signature on SOM, and hence greater rigidity in soil C:N. In contrast, leaf litter C:N affects the C:N of ECM soils, suggesting that soil N is processed somewhat independently of C, as would be the case if ECM fungi "mine" N from SOM. Our findings suggest that biome-wide differences in soil C:N between mycorrhizal types may be due to the interactive effects of litter C:N.

In contrast to leaf litter, it is less clear how root litter C:N affects soil C:N. Although the interaction of mycorrhizal type and root litter C:N was statistically significant (Figure 2), this may be due to the limited range of ECM root litter C:N sampled in our study. When the interaction of mycorrhizal type and root litter C:N was removed from the model and the two terms were treated separately, root litter C:N did not significantly affect soil C:N. In our litter-addition incubation experiment, we found that ECM root litter caused a dampened respiration response compared to AM root litter and that this finding was not related to litter C:N. There may be a similar root litter effect on soil C:N that is not well explained by root litter C:N.

Our findings suggest that either the way that leaf and root litter affect soil C:N is distinct or that it is more appropriate to consider the stoichiometry of the entire litter pool (leaf and root litter together, rather than separately) entering soils. The first possibility is that the relative production of leaf and root litter inputs determines litter effects on soils. The amount of root vs. leaf litter production is highly variable among species and forest types (Vogt et al. 1986a, Vogt et al. 1986b) and these differences along with litter stoichiometry determine the overall quality of organic material entering the decomposer subsystem. It is possible that the tree species we have chosen produce higher quantities of leaf litter than root litter and that is why leaf litter C:N (along with mycorrhizal type) drives soil C:N, but root litter C:N does not (Freschet et al. 2013). A second possibility relates to the depth of these litter inputs. Leaf C:N most influences soil C:N in the soils sampled in our study (0-10 cm), but it is possible that an investigation of soil C:N in deeper soils may reveal a stronger effect of root litter C:N (Schmidt et al. 2011), as roots may become proportionately more important as a source of detritus.

A third consideration in understanding the litter effects on soils is that leaf and root C:N may not be coordinated at the species level, suggesting that the average of leaf and root litter C:N

(at the tree level) may more accurately reflect litter entering the decomposer subsystem. In our study, leaf and root litter C:N were not correlated among species. To investigate this idea further, we analyzed the average of leaf and root litter C:N for each species and found that it to be close to significant ($p=0.09$) for predicting soil C:N (Supplemental figure 1). Collectively, these findings suggest that litter C:N appears to be important in determining soil C:N, but it is not clear if mycorrhizal type plays a mediating role.

Our results suggest that AM and ECM fungi develop microbial communities that are distinct in their makeup and functional attributes. Ectomycorrhizal microbial biomass C:N was higher than AM microbial biomass C:N, regardless of a tree's litter stoichiometry (Figure 6). Microbial biomass C:N can be a proxy for the relative abundance of bacteria or fungi in microbial biomass, since bacteria have a lower C:N than fungi (Deruiter et al. 1993). Our findings lend support to the hypothesis presented by Phillips et al. (2013) that ECM trees lead to the assembly of decomposer communities (including ECM fungi themselves) that are more dominated by fungi relative to those of AM trees.

Soil C:N may be correlated with the ratio of fungi to bacteria. Our study found that microbial biomass C:N is positively correlated with soil C:N (Figure 7). Differences in microbial biomass C:N, or the fungi to bacteria ratio specifically, are correlated with soil C:N across terrestrial ecosystems (Fierer et al. 2009). Therefore, considering our soil C:N and microbial biomass C:N results together lends further support to the idea that AM and ECM soils cycle C and N from litter and SOM in distinct ways. In contrast, our results did not indicate a significant mycorrhizal difference in microbial biomass C:P (Figure 8), largely due to the variability associated with these measurements.

Contrary to our expectations, soil nutrient availabilities were not well explained by mycorrhizal type. Ammonium availability declined with increasing root litter C:N (Figure 3), but was unaffected by leaf litter C:N. This suggests that the C:N of roots, but not leaves, determines the rate of N mineralization in tree-specific soils. The differing effect of root and leaf litter C:N could be caused by spatial differences in the inputs and decomposition of leaf and root litter. While leaf litter is likely to be decomposed in the litter layer (Schmidt et al. 2011), spatially separated from most fine roots, root litter enters directly into mineral soil horizons where N mineralization is dominant (Rasse et al. 2005), which may cause root litter C:N to act as a stronger control on N mineralization. The pattern of DIN availability was the same as ammonium, likely because ammonium is the primary contributor to DIN. Our results show a tendency for higher NO_3^- levels in AM versus ECM soils, although it was not significantly different, largely due to the high variability of NO_3^- in AM soils (Figure 4). Phillips et al. (2013) observed higher rates of nitrification in AM-dominated versus ECM-dominated forests. The lack of concordance between our studies may be due to the fact that spatial patterns in mobile nutrients, such as NO_3^-, are more easily observed at the stand level, rather than at the level of individual trees. Nitrate availability was unaffected by leaf or root litter C:N. Phosphate availability was not different between mycorrhizal types (Figure 5). Collectively, these results suggest that in mixed-mycorrhizal forests, mycorrhizal type does not create major differences in nutrient availability that are discernable at the spatial scale of the individual tree.

Our results demonstrate that soil respiration, a proxy of SOM decomposition, is determined by the interaction of soil C:N and mycorrhizal type. Soil organic matter decomposition is a primary way that C is introduced to the atmosphere and nutrients are recycled in terrestrial ecosystems (Swift 2001), and in our study, we observed differences in the regulation

of this pathway. AM soils support increasing rates of respiration with increasing soil C:N, while ECM soils respire at a consistent rate and are insensitive to soil C:N (Figures 9 and 10). This finding lends further support to the idea that mycorrhizal types support distinct microbial communities, responsible for regulating soil C:N. Arbuscular mycorrhizal-conditioned microbial communities appear to be sensitive to soil C:N, suggesting that they may be compositionally different at varying soil C:N, whereas ECM-conditioned communities may be compositionally similar at varying soil C:N.

The heterotrophic response to litter addition was dependent on litter type (leaf vs. root). Leaf litter addition enhanced soil respiration (relative to the soil-only controls) more than root litter addition, even when litters contained a similar C:N (Figure 11). This result indicates that leaves and roots have different chemical or physical properties that make them more or less available to microbial decomposition (Rasse et al. 2005, Freschet et al. 2013) and that this difference is not captured by litter C:N alone. Other studies have also found different decomposition responses of leaf and root litter in litterbag studies (Vivanco and Austin 2006, Hobbie et al. 2010) and this pattern has been attributed to structural components of roots, particularly lignin (Freschet et al. 2012). In our study, the response of soil respiration was strongly determined by litter C:N across all treatments, where respiration declined with increasing litter C:N likely due to microbial N limitation when litter C:N is high (Melillo et al. 1982, Taylor et al. 1989, Aerts 1997). Interestingly, for the leaf litter addition effect on soil respiration, there was no effect of soil mycorrhizal type or litter mycorrhizal type and only a weak trend for litter C:N (Figure 12 and 13).

For root litter additions, soil mycorrhizal type, litter mycorrhizal type, and litter C:N all individually affected the response of soil respiration (Figure 14 and 15). Although litter

mycorrhizal type and litter C:N both significantly affected the response of soil respiration, these two terms were not correlated, that is, the mean root litter C:N was not different between mycorrhizal types (Table 2). Also, the response of respiration was not well explained by any measure of mycorrhizal colonization for either AM or ECM roots (Table 3). This suggests that there is something fundamentally different about ECM roots that makes them resistant to degradation (Robinson et al. 1999), beyond root litter C:N or mycorrhizal colonization. This finding lends support to the hypothesis of Langley and Hungate (2003) that ECM roots are decomposed at a slower rate than AM roots, but calls into question the suggested mechanism of ECM mycorrhizal colonization behind this observation. Fernandez and Koide (2014) showed that the melanin in ECM fungal cell walls slowed decomposition, suggesting that the presence of melanin in fungal structures may be suppressing decomposition amongst the ECM roots in our study. We also observed a soil mycorrhizal effect in the root litter additions. Ectomycorrhizal soil respiration was more stimulated by root litter inputs than was respiration in AM soils. Furthermore, ECM roots suppressed respiration in AM soils compared to the soil-only controls, but not in ECM soils. This suggests that ECM decomposers have a greater functional breadth (Keiser et al. 2014) than AM decomposers in that they are capable of decomposing a broader range of organic material.

A potential source of error in this experiment is the narrow range of ECM root litter C:N represented by our tree species. Root litter C:N interacted with mycorrhizal type to determine soil C:N and ammonium and DIN availability. Although these interactions are statistically significant, it is unclear if the response is biologically meaningful. It may require greater sampling, across a broader range of ECM root litter C:N, to determine this.

Another concern is the limitation of the metric of C:N to explain microbial community makeup and litter quality. Microbial biomass C:N is a useful proxy for determining the ratio of fungi to bacteria, but DNA sequencing data would further elucidate differences between microbial community structure in AM and ECM soils. Litter quality was not totally explained by litter C:N. Though leaf and root litter had overlapping C:N (Figure 11), leaf litter caused a greater respiration response than root litter, indicating differences in lability.

In a mixed-mycorrhiza forest like Whitehall forest, it is likely that many AM and ECM root systems overlap and that root litter inputs mix within this context. Due to this spatial complexity, mycorrhizal and root litter effects are likely to be strongest close to the stem of a given tree and may decline with increasing distance from the stem. Because of this, it may be useful to consider diameter at breast height (DBH) of trees, as a proxy of tree-mediated effects on soils, to better predict how trees affect biogeochemical patterns. In contrast, leaf litter effects may be highly variable because of the potential for leaves to be blown to far distances from the source tree, and therefore mixed with leaves of other tree species. A pressing question is if there is a difference in root vs. leaf litter production among species and mycorrhizal types as this could fundamentally alter our view of the total input to the decomposer subsystem (Vogt et al. 1986b).

Major changes are predicted for the assemblages of tree species making up temperate forests (Iverson et al. 2008) due to species migration in response to climate change and species losses and subsequent replacement in response to pests and pathogens (Lovett et al. 2010). Therefore, understanding the effects of mycorrhizal type and litter stoichiometry on soil biogeochemistry may allow for more accurate predictions of future terrestrial C and N cycles. For example, if an ECM tree replaces an AM tree, soil respiration may decrease as ECM fungi begin to mediate a newly fungal-dominated microbial community and ECM root litter begins to

suppress decomposition. In this way, changes in a forest's assemblage could diminish or enhance net sink capacity of C. As a specific example, tree-species abundance models for southeastern North America suggest that AM Tulip poplar (LITU) may decrease its current range as ECM White oak (QUAL) increases in abundance (Iverson et al. 2008). In areas where this replacement occurs, an increase in soil C storage could be expected. Additionally, soil C:N of forest soils is an accurate indicator of the broad-scale process of watershed nitrate leaching (Lovett et al. 2002, Lovett et al. 2004), so the predictive abilities of tree species mycorrhizal type and litter stoichiometry go beyond ecosystem C storage.

Our results support previous findings that mycorrhizal type can influence biogeochemistry of tree soils, but provides new mechanistic evidence that AM and ECM trees give rise to functionally distinct microbial communities and that some mycorrhizal effects manifest as a function of litter stoichiometry. Our study demonstrates that mycorrhizal type and litter stoichiometry must be considered in tandem in order to make predictions about soil biogeochemical characteristics and offers insight into what parameters best predict several factors. Finally, our study offers predictive indicators of biogeochemical consequences of tree species migration and replacement.

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SUPPLEMENTAL TABLE 1

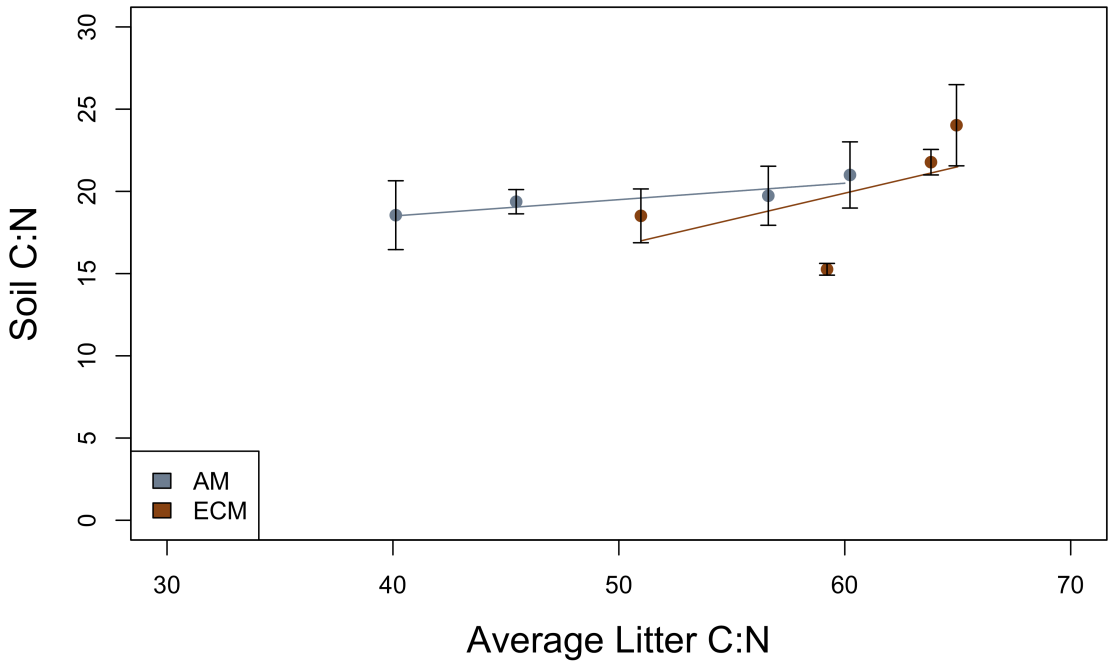
Mixed effect model constructions. All mixed models were created in R using the lme4 package. For each model construction, the first thing listed is the dependent variable. Everything after the tilde (~) is an independent variable. Interactions are denoted by an asterisk (*) between two terms. Random effects are expressed in parentheses. The abbreviation "Myc" refers to mycorrhizal type. Carbon to nitrogen ratio is expressed as C:N. All p-values were calculated using the likelihood ratio test. The estimate, standard error, χ^2 value with the associated degrees of freedom and p-value is listed for each fixed effect.

Model construction: Soil C:N~Myc*Leaf C:N + Myc*Root C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc*Leaf C:N	0.30	0.15	4.21	0.04
Myc *Root C:N	-0.53	0.24	5.38	0.02
Model construction: Soil C:N~Myc*Leaf C:N + Root C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc*Leaf C:N	0.30	0.20	3.68	0.06
Root C:N	0.02	0.06	0.24	0.63
Model construction: Soil C:N~Myc*Average C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc*Average C:N	0.26	0.28	1.79	0.18
Model construction: Soil C:N~Myc + Average C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc	-1.30	2.10	0.57	0.45
Average C:N	0.18	0.13	2.88	0.09
Model construction: MB C:N~Myc+ Soil C:N + Leaf C:N + Root C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc	2.44	1.37	4.14	0.04
Soil C:N	0.23	0.12	3.29	0.07
Leaf C:N	-0.01	0.07	0.01	0.55
Root C:N	-0.01	0.04	0.06	0.81
Model construction: MB C:P~Myc + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**

Myc	340.4	399.5	0.91	0.34
Model construction: Nitrate~Myc +Leaf C:N + Root C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc	-0.01	0.01	1.00	0.32
Leaf C:N	-0.0001	0.0006	0.03	0.86
Root C:N	-0.0002	0.0004	0.45	0.50
Model construction: Ammonium ~ Myc * Root C:N + Leaf C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc*Root C:N	0.10	0.05	3.60	0.06
Leaf C:N	-0.01	0.02	0.63	0.43
Model construction: Ammonium ~ Myc + Root C:N + Leaf C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc	-0.07	0.38	0.05	0.83
Leaf C:N	-0.01	0.02	0.16	0.69
Root C:N	-0.02	0.01	2.89	0.09
Model construction: DIN ~ Myc * Root C:N + Leaf C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc*Root C:N	0.11	0.05	0.74	0.39
Leaf C:N	-0.01	0.02	4.11	0.04
Model construction: DIN ~ Myc + Root C:N + Leaf C:N + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc	-0.06	0.39	0.03	0.85
Leaf C:N	-0.01	0.02	0.19	0.66
Root C:N	-0.02	0.01	2.88	0.09
Model construction: Phosphate~Myc + (Species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc	-0.03	0.06	0.29	0.59
Model construction: CO_2 Efflux ~ Soil myc * Soil C:N + (Soil species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Soil myc*Soil C:N	-362	156	6.82	0.01
Model construction: (all litter additions) CO_2 Deviation~Litter Type+Litter C:N + (Soil species) + (1|Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Litter Type	-2128.53	166.65	136.33	<0.001
Litter C:N	-34.98	7.61	20.70	<0.001
Model construction (leaf litter additions): CO_2 Deviation ~ Soil myc + Litter myc + Litter C:N + (Soil species) + (Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Soil myc	-86.96	565.11	0.03	0.86
Litter myc	-482.90	851.18	0.48	0.49
Litter C:N	-27.31	39.45	0.70	0.40
Model construction (root litter additions): CO_2 Deviation ~ Soil myc + Litter myc + Litter C:N + (Soil species) + (Litter species)				

Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Soil myc	462.75	283.21	2.81	0.09
Litter myc	-803.74	285.01	7.23	0.01
Litter C:N	-26.51	10.32	6.38	0.01
Model construction (ECM roots only): CO_2 Deviation ~ Myc colonization (grid method) + (Soil species) + (Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc colonization	-412.45	3550.87	0.02	0.88
Model construction (ECM roots only): CO_2 Deviation ~ Myc colonization (tip method) + (Soil species) + (Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
Myc colonization	-45.22	778.00	0.003	0.96
Model construction (AM roots only): CO_2 Deviation ~ % Arbuscules + (Soil species) + (Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
% Arbuscules	2779.9	2411.3	1.90	0.17
Model construction (AM roots only): CO_2 Deviation ~ % Vesicles + (Soil species) + (Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
% Vesicles	943.0	1356.2	0.81	0.37
Model construction (AM roots only): CO_2 Deviation ~ % Hyphae + (Soil species) + (Litter species)				
Effect	**Estimate**	**Standard error**	$\chi^2_{(1)}$	**p-value**
% Hyphae	894.8	2293.0	0.28	0.60

SUPPLEMENTAL FIGURE 1



Supplemental Figure 1: The relationship between soil C:N and the average litter C:N (leaf and root) produced by each tree species. The interaction of mycorrhizal type with average litter C:N is not significant (p=0.18), but average litter C:N is close to significant (p=0.09). Values represent means and standard error (SE) of soil C:N.