

**Plant-microbe interactions influence ecosystem processes: the roles of roots and
mycorrhizal fungal hyphae on terrestrial carbon dynamics**

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DEDICATION

I dedicate my dissertation to my husband, Adam Moore, and our children, Noah and Silas, for their consistent encouragement, enduring patience, enlightening questions, and genuine interest in my scientific passion.

My pursuit of science is dedicated to my mother, who always said that women in science is a recipe for success.

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ABSTRACT

Plant-microbe interactions shape ecosystem processes such as productivity and decomposition of organic matter. Plants interact with mycorrhizal fungal hyphae to acquire nutrients from soil in exchange for plant-assimilated carbon. The mycorrhizal interaction is therefore a key influence on ecosystem carbon dynamics. Mycorrhizal fungi are key players in soil carbon cycling as they stimulate plants to allocate carbon belowground, and mycorrhizal fungal hyphae interact with microbial decomposers of soil carbon. However, there are few studies on mycorrhizal fungal hyphal interactions with roots and soil organisms in light of soil carbon accrual and release, important ecosystem processes. In my doctoral research, I examined interactions among plants, mycorrhizal fungal hyphae, and soil microbes in order to better understand the role of mycorrhizal fungal hyphae in soil C dynamics through combining models with experiments. In my first chapter, I discussed the direct role of mycorrhizal fungi on organic matter decomposition and subsequent shifts in soil carbon. I compared the effect of mycorrhizal hyphal decomposition to decomposition by free-living microbes using a carbon simulation model. In my second chapter, I tested how roots and mycorrhizal fungal hyphae indirectly affect decomposition of organic matter through interactions with free-living microbes. I found non-additive effects of roots and mycorrhizal fungal hyphae on soil microbial activity and hypothesized the effects were driven by nutrient demand. Thus, in my third chapter, I examined microbial decomposition of carbon and nutrient substrates across a range of root and mycorrhizal hyphal influence. My dissertation advances the field of ecosystem ecology by evaluating the role of plant-microbe interactions in soil carbon dynamics.

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Introduction

Biological interactions in soil influence ecosystem processes such as primary productivity, nutrient cycling, and decomposition (van der Heijden et al. 2008, Bardgett and Wardle 2010). One biological interaction important to the balance of carbon (C) in terrestrial ecosystems is the plant-mycorrhizal interaction (Cheng et al. 2012). Thus understanding the links between mycorrhizal and plant root function and soil processes is a major theme in ecosystem ecology, but one that remains poorly understood.

Mycorrhizal fungi play diverse roles in soil C cycling (Antoninka et al. 2009, Grey et al. 2011). Mycorrhizal fungi act as C sinks in soils as they use C assimilated by a host plant to build biomass (Lopez et al. 2007, Wiemken 2007). Their secretions also increase soil aggregation, in turn increasing soil C accumulation (Tisdall et al. 1997). Their activity, however, can directly and indirectly lead to the breakdown of soil C (Read and Perez-Moreno 2003, Cheng et al. 2012). Some guilds of mycorrhizal fungi secrete enzymes to break down organic substrates in soil and use the C and nitrogen (N) they attain to build amino acids (Talbot et al. 2008). Mycorrhizae also have indirect effects on soil C stocks by stimulating decomposition by other soil microbes (Cheng et al. 2014). Alternatively, mycorrhizal fungi can suppress activity of saprotrophic microbes either through use of antibiotics or through competitive exclusion (Orwin et al. 2011). Thus, mycorrhizal fungi may at times promote soil C storage and at other times promote the release of soil C back to the atmosphere as CO₂. The net effect of mycorrhizal fungi depends on environmental conditions, the soil community, soil type, and chemical complexity of organic substrates in the soil (Moore et al. 2015, Donnelly et al. 2004, Read et al. 2004, Agerer 2001). However, we have limited understanding of when, where, and why these switches between C sink and source occur, which inhibits our ability to accurately predict future C scenarios.

Understanding the processes that regulate soil C storage and release to the atmosphere will enable us to better predict global changes that are contingent upon C dynamics (Todd-Brown et al. 2012). The most widely used C models describe decomposition rates as a constant function (Parton et al. 1998, Parton et al. 1987, Jenkinson and Raynor 1977). These models typically overestimate stocks of soil C

(Smith et al. 1997), and it's thought that adding explicit soil microbial activity to the model structure will improve model-data fit (Treseder et al. 2012, Schimel 2013). Soil C decomposition leads to a flux of C from soil to the atmosphere, which is mediated by members of the soil community including arthropods (Wickings et al. 2012), bacteria (Clarholm 1985), free-living fungi (Baldrian and Lindahl 2011), and mycorrhizal fungi (Cheng et al. 2012). Because soil organisms drive C decomposition, changes in their activity could alter soil C storage (Wallenstein and Hall 2012). We can more confidently predict soil C feedbacks to the atmosphere by understanding how interactions within the rhizosphere alter C dynamics (Schimel 2013).

The following chapters of my dissertation detail my research on direct and indirect effects of plant-mycorrhizae interactions on soil microbial decomposition and ecosystem C pools and fluxes. I examined direct effects of mycorrhizal fungi on decomposition of organic matter using a simulation C model by modifying theory developed for free-living microbes (Lawrence et al. 2009) and applying it to mycorrhizal fungi. I explored two alternative mechanisms of mycorrhizal decomposition (Talbot et al. 2008) to determine their relative influence on soil C storage. Next, I investigated indirect influences of roots and mycorrhizal fungi on microbial decomposition in a field experiment. I manipulated root and mycorrhizal access to the soil microbial community and measured pools of C, soil respiration, and microbial C-degrading enzyme activity. I scaled up field-measured C fluxes to determine how roots and mycorrhizal fungi affect long-term C pools using an ecosystem model. The results from that study led me to wonder how roots and mycorrhizae interact with soil microbes differently to affect C and nutrient-degrading activity. In a follow-up field experiment, I examined microbial activity related to decomposition of organic matter specific to C and nutrients across a range of root and mycorrhizal influence. Overall, I found that roots and mycorrhizal fungi increased decomposer activity and that C-degrading activity responds to roots while N-degrading activity responds to mycorrhizal fungi. Below I outline how my dissertation research filled a knowledge gap in ecosystem ecology by showing that soil C decomposition is contingent upon root-mycorrhizae-microbial interactions.

Decomposition by ectomycorrhizal fungi alters soil carbon in a simulation model

The amount of C stored in soil depends on the rate that soil microbes decompose organic matter and mineralize C. Many microbes in soil, including mycorrhizal fungi, gain energy from decaying organic matter. The trophic strategy of ectomycorrhizal fungi (ECM) varies along a continuum from biotrophic to saprotrophic (Koide et al. 2008). While they are typically biotrophic, mycorrhizae can act as saprotrophs, obtaining soil C using extracellular enzymes similar to those used by decomposer bacteria and free-living fungi (Read & Perez-Moreno 2003). In addition to simple sugars, ECM can decompose more complex organic matter like plant cell wall components (e.g., cellulose and hemicellulose) (Vaario et al. 2011) and lignin (Read and Perez-Moreno 2003). For example, *Suillus granulatus* growing with *Pinus contorta* produces ligninolytic enzymes to degrade wood (Cullings et al. 2008). Decomposer activity varies among ECM species, with some species capable of three times greater C-degrading activity than others (Tedersoo et al. 2012). While ECM saprotrophy has been observed, the mechanisms behind the activity are still unclear.

Talbot et al. (2008) proposed two hypothetical mechanisms for saprotrophic activity in ectomycorrhizal fungi, a mycorrhizal guild with documented decomposition capabilities (Read & Perez-Moreno 2003). One hypothesis is that ectomycorrhizal fungi breakdown organic matter and acquire C from it at a constant rate. An alternate hypothesis is that mycorrhizae respond to plant stress (i.e., reduced photosynthesis) by increasing their rate of mass-specific enzyme activity to gain access to soil C. To examine these alternative mechanisms, I developed a simple C flux model to simulate the effect of mycorrhizal decomposition on soil C stocks and compared the two mechanisms proposed by Talbot et al. Models such as this, where enzyme activity rates fluctuate, have been developed for microbial enzymatic processes (Schimel and Weintraub 2003, Moorhead and Sinsabaugh 2006, Allison 2012, Wang et al. 2012). My model was one of the first to apply microbial enzymatic theory to mycorrhizal activity in soil (but see, Deckmyn et al. 2008, Meyer et al. 2010, Orwin et al. 2011).

To test the first hypothesis, mycorrhizal decomposition activity remained constant and independent from plant C allocation. As a result, C storage was most reduced under low levels of plant stress (i.e., high photosynthesis and belowground C allocation). This seemed to be because mycorrhizal biomass, and thus total enzyme production, was highest. The effect of mycorrhizal fungi on decomposition of soil C was stronger when mycorrhizae responded to plant stress by changing their rate of decomposition. Using an alternative model where mycorrhizal fungi adjusted rates of decomposition activity in response to changes in plant stress, I found that soil C storage decreased by 3% when plants were stressed. This effect was smaller than anticipated, likely because mycorrhizae used C more efficiently when it was derived from a plant than from soil. This makes sense because mycorrhizae use relatively labile C compounds from plants, whereas soil C is locked in larger polymers. Given soils store 500 – 3000 Pg C globally, which is more than the atmosphere and all vegetation combined (Todd-Brown et al. 2013), even a 3% fluctuation can greatly impact the global C budget. This work demonstrated the importance of direct effects of mycorrhizal fungi on decomposition of organic matter and soil C stocks.

Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes

Understanding drivers of microbial decomposition of organic matter is critical for understanding C stabilization and release in soil, and ultimately making predictions about carbon-climate feedbacks. Microbial activity is shaped by the microbes' interactions with other members of the soil community. Roots increase microbial activity through priming effects, exuding simple sugars that provide microbes with energy to build complex enzymes used to break down organic matter (Kuzykov 2010). We must also consider interactions between microbes and mycorrhizal fungi because they occupy the same space and use similar resources. However, we know little about the effects of mycorrhizae on microbial activity. Mycorrhizae may prime microbial activity in a similar way as roots through exudation. ECM secrete polysaccharides into the soil to “prime” other members of the soil community to decompose C compounds (Frey-Klett et

al. 2007, Toljander et al. 2007). Because microbes are typically C-limited, an increase in C to the soil could stimulate microbes to produce complex enzymes to degrade complex soil organic matter (SOM), like lignin (Nazir et al. 2010). The aim of this work was to determine how roots and mycorrhizae affect microbial decomposition of organic matter. I expected mycorrhizae to amplify priming effects of roots on microbial activity. To address this question, I experimentally manipulated root and mycorrhizal access to soil within PVC cores using mesh of varying opening size. I used a ^{13}C -labelled substrate to trace C from soil organic matter into respiration and measured microbial extracellular enzyme activity.

This experiment showed roots and mycorrhizae affected microbial activity differently, which has implications for the way we model C dynamics. Enzyme activity was two to three times higher when roots and mycorrhizae were either simultaneously excluded or both present compared to when roots were excluded and mycorrhizae are present. This finding suggested mycorrhizae may compete with microbes for nutrients or space in the soil matrix and thus inhibit their activity. Root presence, however, dampened mycorrhizal effects on microbes, possibly through priming. I found that microbes utilized root and mycorrhizal exudates as a C source, but when both were excluded microbes used easily degradable SOM. My findings suggested that, in contrast to my expectations, mycorrhizae had different effects on microbes near compared to far from roots (i.e., in cores excluding roots compared to cores including roots). Mycorrhizae seemed to inhibit microbial enzyme activity far from roots. In conclusion, roots and mycorrhizae affected microbial activity thereby altering fluxes of C from soil to atmosphere. Therefore, mycorrhizal fungi may be a key biological component currently missing from ecosystem models.

I furthered this work by testing whether inclusion of mycorrhizae into C models could alter simulated pool sizes. I compared the effect of roots and mycorrhizal fungi on soil C using an ecosystem model. The Microbial ENzymatic Decomposition (MEND) model was developed to analyze effects of different types of C-degrading enzymes on soil C pools and CO_2 respiration (Wang et al. 2013). Given my field experiment

demonstrated that roots and mycorrhizal fungi have variable effects on C-degrading enzymes, I tested the different functions of roots and mycorrhizal fungi using the MEND model. In the model, complex C-degrading enzymes (i.e., oxidative enzymes) were linked to a slowly turning over pool of C, while labile C-degrading enzymes (i.e., hydrolytic enzymes) were linked to a quickly turning over pool. The simulations revealed that soils without root and mycorrhizal influences stored more C compared to soils with roots and mycorrhizal influences. The most C was stored in soils influenced by mycorrhizal fungi but not roots in a pool of particulate organic C (larger fragment size relative to mineral organic C). We suspect this is due to competitive interactions between mycorrhizal fungi and free-living decomposer microbes. Other models have similarly shown soil C stocks increase when mycorrhizal fungi suppress the activity of free-living soil microbes (Orwin et al. 2011). This may also be due to mycorrhizal tissues turning over slowly and thus contributing to soil C accrual. Some evidence suggests mycorrhizal roots decay more slowly than non-mycorrhizal roots (Langley et al. 2006). In sum, this work demonstrated the indirect effect of roots and mycorrhizal fungi on soil C-degrading activity of microbes and their influence on different pools of soil C.

Roots stimulate soil carbon decomposition while mycorrhizal hyphae stimulate microbial nutrient acquisition

Roots and mycorrhizal fungi influence soil microbes directly and indirectly, but the relative strengths of those effects are poorly understood. Roots have a direct and stimulating effect on soil microbial activity by releasing C-rich exudates. This effect, known as microbial “priming” (Kuzyakov 2010), can change rates of organic matter decomposition (de Graaff et al. 2010). Mycorrhizal fungi have direct effects on soil microbes by competing for access to space and shared resources (de Boer 2005). Either roots or fungi could have indirect effects on soil microbial activity by changing the microbial community (Nuccio et al. 2007). The direct effects of roots and mycorrhizal fungi and their relative strength compared to indirect effects on soil microbes is a knowledge gap.

I tested the relative strength of direct and indirect effects of roots and mycorrhizal fungi on microbial biomass and activity using *in situ* manipulations of biomass. I used mesh-lined cores buried in soil to create a gradient of root and mycorrhizal biomass in soil. I measured root, mycorrhizal and microbial biomass and indicators of microbial activity (extra-cellular enzymes, soil respiration, metabolism of ^{13}C -labeled starch). I found that the direct effects of roots and mycorrhizal fungi on microbial enzymes was stronger than their indirect effects that are mediated by changes in microbial biomass. Roots increased C-degrading enzyme activity while mycorrhizal fungi decreased N-degrading enzyme activity, and these indirect effects were stronger than the direct effects. Metabolism of ^{13}C -starch matched the response of N-degrading enzyme activity. Altogether, these results corroborate theory developed by others suggesting that mycorrhizal fungi suppress microbial activity in effort to compete for soil N (Orwin et al. 2011). This study has implications for the way we model ecosystem C fluxes, which are currently represented as direct functions of changes to microbial biomass (Lawrence et al. 2009, Todd-Brown et al. 2013). I showed that enzyme activity, and therefore rates of decomposition, are a function of root and mycorrhizal biomass in addition to microbial biomass.

Chapter 1: Decomposition by ectomycorrhizal fungi alters soil carbon storage in a simulation model

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Abstract

Carbon cycle models often lack explicit belowground organism activity, yet belowground organisms regulate carbon storage and release in soil. Ectomycorrhizal fungi are important players in the carbon cycle because they are a conduit into soil for carbon assimilated by the plant. It is hypothesized that ectomycorrhizal fungi can also be active decomposers when plant carbon allocation to fungi is low. Here, we reviewed the literature on ectomycorrhizal decomposition and we developed a simulation model of the plant-mycorrhizae interaction where a reduction in plant productivity stimulates ectomycorrhizal fungi to decompose soil organic matter. Our review highlights evidence demonstrating the potential for ectomycorrhizal fungi to decompose soil organic matter. Our model output suggests that ectomycorrhizal activity accounts for a portion of carbon decomposed in soil, but this portion varied with plant productivity and the mycorrhizal carbon uptake strategy simulated. Lower organic matter inputs to soil were largely responsible for reduced soil carbon storage. Using mathematical theory, we demonstrated that biotic interactions affect predictions of ecosystem functions. Specifically, we developed a simple function to model the mycorrhizal switch in function from plant symbiont to decomposer. We show that including mycorrhizal fungi with the flexibility of mutualistic and saprotrophic lifestyles alters predictions of ecosystem function.

Key Words: carbon cycle; decomposition; ectomycorrhizae; extracellular enzyme activity; plant-soil interactions; simulation model; soil carbon sequestration.

Introduction

While interactions among species shape community structure and ecosystem function, they also increase ecosystem complexity and thus model complexity. However, recent calls for improving soil decomposition models highlight the importance of incorporating biological mechanisms that lead to ecosystem fluxes (Treseder et al. 2012, Schimel 2013, Wieder et al. 2013, Xu et al. 2014). For example, decomposition of soil organic matter by microbes through extracellular enzymes and subsequent mineralization transforms carbon (C) stored in soil into atmospheric C, a flux that has ramifications for greenhouse gas levels and thus climate. Models linking soil and atmospheric pools of C have recently advanced because of our increased understanding of microbial ecology (Treseder et al. 2012). Field measurements of decomposition closely match model predictions when we explicitly model microbes with diverse traits (Allison 2012) and guilds growing at various rates and specializing on different types of organic matter (Moorhead and Sinsabaugh 2006). Functional groups of microbes, such as free-living and mycorrhizal fungi, respond differently to soil nutrient availability and modeling different groups of fungi changes predictions of ecosystem nutrient cycling (Orwin et al. 2011). However, most ecosystem models still do not incorporate distinct microbial groups, even though their roles in ecosystem processes are diverse.

Interactions among species can influence important ecosystem functions such as net primary productivity and decomposition (Bruno et al. 2003, van der Heijden et al. 2008). For example, mycorrhizal fungi form a mutualism with one or several plants (van der Heijden and Horton 2009; Philip et al. 2010) where soil nutrients are exchanged for plant-assimilated C. Mycorrhizal plants transfer 23% more assimilated C belowground compared to non-mycorrhizal plants (Rygielwicz and Andersen 1994) thus altering soil C storage. Carbon transfer to mycorrhizae varies with plant genotype and species (Hoeksema and Classen 2012), season (Apple et al. 2005, Courty et al. 2007), and soil nutrient availability (Olsson et al. 2010). Carbon transferred to mycorrhizal fungi can contribute to soil C storage if fungal tissues decompose more slowly than fine roots (Langley et al. 2006) or if their biological residues persist for decades to centuries (Clemmensen et al. 2013, Cotrufo et al. 2013). Mycorrhizal fungi can also slow

decomposition by monopolizing humus layers and reducing activity and abundance of free-living decomposers (Lindahl et al. 2010, McGuire et al. 2010, Orwin et al. 2011). Finally, mycorrhizal fungi may increase soil organic matter stabilization by increasing soil aggregation (Daynes et al. 2012).

On the other hand, mycorrhizae may contribute to soil C release if fungal tissues decompose more quickly than fine roots (Fernandez and Koide 2011, Koide et al. 2011), if they are exuding labile carbon sources, or if they are actively decomposing organic matter (Read and Perez-Moreno 2003). In this paper we review mechanistic hypotheses of ectomycorrhizal (ECM) decomposition activity and then discuss which, where, and when mycorrhizae might decompose organic matter in soil. Because mycorrhizae are part of the soil microbial community and have some potential decomposition capabilities, we highlight the importance of recognizing mycorrhizal fungi as a distinct group in a conceptual C model. We present a conceptual model that demonstrates how ECM decomposition of soil carbon may influence soil carbon dynamics and then use the model to compare two alternative mechanistic scenarios of when mycorrhizal fungi may take part in soil carbon decomposition.

Evidence for saprotrophic activities of ECM

The morphology and enzymatic capabilities of ECM make them more likely to decompose organic matter than arbuscular mycorrhizal fungi (Chalot and Brun 1998, Read and Perez-Moreno 2003, Talbot et al. 2008). ECM produce prolific belowground structure of mycelium, thus ECM are more likely than arbuscular mycorrhizae to decompose organic matter. Additionally, ECM exude extracellular enzymes that AM fungi do not produce to decompose organic matter (Norkrans 1950, Abuzinadah and Read 1986, Smith and Read 2008, Bodeker et al. 2009) including cellulose (Chalot and Brun 1998, Vaario et al. 2012) and lignin (Read and Perez-Moreno 2003, Bodeker et al. 2009). Finally, ECM are commonly found proliferating in patches of organic matter such as decomposing nematode biomass (Perez-Moreno and Read 2001) and decaying logs (Austin 2013). While decomposition of soil C by ECM seems likely given observed

enzyme activities and their abundance in patches of organic matter, we are still unable to predict which ECM species might act as decomposers.

ECM, even within a genus, vary in having genes that indicate the ability to decompose organic matter. This may be because the genes that code for decomposition, saprotrophy, have evolved and been lost several times across ECM phylogenies (Tedersoo et al. 2010). Having the genetic ability to degrade organic matter does not necessarily translate to using the function, however. For example, while some *Amanita* species lack genes coding for organic matter degradation (endoglucanase and cellobiohydrolase) others retain similar C-degrading genes (e.g., beta-glucosidase). Select *Amanita* species grown in culture cannot survive saprotrophically given organic nutrients (Wolfe et al. 2012); yet, other unculturable ECM genera are suspected of decomposition including *Cortinarius* (Bodeker et al. 2009), *Suillus*, *Rhizopogon*, *Cennococcum*, *Laccaria* (Durall et al. 1994), *Lactarius* (Courty et al. 2007, Bodeker et al. 2009) *Paxillus* (Chalot and Brun 1998, Perez-Moreno and Read 2001, Rineau et al. 2012), *Russula* (Bodeker et al. 2009), and *Tomentella* (Courty et al. 2010). Clearly, research needs to target other species of ECM using functional gene assays to predict which mycorrhizal taxa are capable of decomposing organic matter.

Alternative mechanisms for ECM decomposition

In a provocative idea paper, Talbot and colleagues (2008) proposed mechanisms for ECM decomposition; we compare two of them here. On one hand, ECM may increase enzymatic activity in response to lower plant C assimilation. When plants allocate less C to ECM, either due to budbreak or dormancy, relative activity of C-degrading enzyme activity increases when mycorrhizae are present than absent (Courty et al. 2007, Cullings et al. 2008). Thus, mycorrhizal decomposition of soil C could be triggered by reduced total allocation of C from the plant to ECM during a prolonged suspension of photosynthesis. Alternative evidence from nitrogen fertilization studies, where relative C allocation to mycorrhizae is reduced with fertilization, shows the number of ECM root tips decreases (Pritchard et al. 2014) while tissues growing away from roots such as rhizomorphs are unaffected (Treseder et al. 2006). These combined results suggest that

when fertilization limits C allocation, mycorrhizae are capable of continuing rhizomorph production via C from an alternative source. Upon regaining adequate supplies of photosynthate, mycorrhizae may stop decomposing soil C. On the other hand, ECM may decompose soil C continuously while breaking down organic matter to obtain nutrients like nitrogen or phosphorus (Rineau et al. 2012). As ECM decompose organic matter and acquire nutrients, which is widely accepted as the basis for the plant-mycorrhiza symbiosis, they could also acquire soil-derived C released during decomposition. It is unknown whether ECM use soil C as an energy source or if they coincidentally acquire C while acquiring N (e.g., from amino acids) (Talbot et al. 2008). Although it is recognized that plant-mycorrhizal interactions affect ecosystem soil C storage, the symbiosis is seldom included in soil decomposition or ecosystem models (but see Deckmyn et al. 2008, Meyer et al. 2010, Orwin et al. 2011).

Similar to ECM, free-living soil microbes decompose soil using extra-cellular enzymes and their activity has recently been made explicit in ecosystem models. While traditional C models account for microbial C mineralization indirectly (e.g., Parton et al. 1988, Thornton and Rosenbloom 2005), recent advances in C models use enzyme kinetics to describe decomposition (Moorhead and Sinsabaugh 2006, Lawrence et al. 2009, Allison 2012, Wang et al. 2012). In models utilizing enzyme kinetics theory, decomposition is described by a logarithmic curve that increases with substrate (i.e., reaction site) availability and plateaus at a maximal enzyme activity rate. Since mycorrhizal fungi and free-living microbes use extracellular enzymes to degrade organic matter, we took an approach similar to the microbial enzyme kinetics models to describe mycorrhizal enzyme activity. To our knowledge, our model is the first to focus on mycorrhizal C acquisition strategies and how these changes influence soil C dynamics. We simplified and focused our model on C dynamics so it could provide a straightforward framework for future experimental tests.

While ectomycorrhizal fungi have the enzyme capacity to degrade soil C (Read and Perez-Moreno 2003, Brzostek and Finzi 2011, Burke et al. 2011, Jones et al. 2012, Vaario et al. 2012), there have been few empirical studies to explore if mycorrhizal fungi directly increase soil C degradation. Theoretical models can be the first step in exploring

how the presence of mycorrhizae might or might not alter soil C dynamics. Here, we developed a theoretical model to explore: (1) how ectomycorrhizal fungi alter soil C dynamics when they uptake C from the soil, (2) if changes in plant productivity alter ectomycorrhizal C uptake from the soil, and (3) if shifts in soil organic matter degradation are due to the direct uptake of C by mycorrhizae or to the response of other soil organisms to ectomycorrhizal activity.

Methods

We developed a simulation model to investigate impacts of mycorrhizal C acquisition strategies on soil C storage. We studied three strategies of mycorrhizal uptake of C that range from C uptake primarily from plants to uptake from plants and soil. In the first strategy, ECM only acquire C from a plant, assuming a constant allocation coefficient. In the second and third strategies they acquire additional C from soil. The second strategy simulates specific ectomycorrhizal enzyme activity rate (i.e., activity per unit mycorrhizal biomass) at a constant rate, whereas in the third strategy the activity rate from soil is variable and depends on substrate availability. We use the enzyme kinetic function (Michaelis-Menten 1913, as cited in Johnson and Goody 2011), which regulates extracellular enzyme activity based on the amount of available substrate. Parameter values are provided (Table 1).

Model description

Carbon moves from aboveground, where it is assimilated into plant biomass, to belowground, where it is decomposed (Fig. 1.1). Plants (P) acquire C from the atmosphere and assimilate it into biomass through net primary production (G) (Eq. 1).

$$dP / dt = Gs - P[a_M + a_R + a_L] \quad \text{Eq. 1}$$

The rate of production is scaled (s) from 0 - 100% as a way to reduce plant productivity, thus reducing the amount of C transported belowground. Environmental factors vary mechanistically in how they alter allocation patterns. We do not focus on mechanisms of abiotic factors that impact allocation patterns, but instead on whether alternative carbon uptake strategies of mycorrhizae can affect soil C storage. Some photosynthate is

allocated to mycorrhizal fungi (a_M) and roots (a_R) while some contributes to leaf production (a_L). The remaining plant biomass can be considered woody tissue or C reserves that will be used during bud-break the following year. Leaf litter (L) accumulates and turns into humus as it enters soil at the rate k_L (Eq. 2).

$$dL / dt = a_L P - k_L L \quad \text{Eq. 2}$$

Similarly, roots (R) grow as plants allocate C to them, and when they turn over they contribute to soil organic matter (k_R) (Eq. 3).

$$dR / dt = a_R P - k_R R \quad \text{Eq. 3}$$

Soil organic matter accumulates as root litter, leaf litter, and decaying mycorrhizal (k_M) and free-living microbial biomass (k_B) enters soil. (Eq. 4).

$$dS / dt = k_L L(1 - r_L) + k_R R + k_M M + k_B B - V_i - V_B \quad \text{Eq. 4}$$

This pool contains labile organic C and is analogous to a “fast”-cycling pool (sensu Parton et al. 1988). We have not modeled a “slow”-cycling pool of C because it is likely that inputs from plants, whether high or low chemical quality, do not directly contribute to a stabilized pool of soil C (Schmidt et al. 2011, Cotrufo et al. 2013). Heterotrophic respiration (r_L) occurs as litter is consumed by litter detritivores. Mycorrhizae take up soil C (V_i), which we modeled in the three strategies as described below. V_B is soil C uptake by free-living (i.e., non-mycorrhizal) microbes.

We simulated mycorrhizal C uptake from soil using three different strategies. First we assumed mycorrhizae do not take up any C from soil (Eq. 5).

$$V_i = 0 \quad \text{Eq. 5}$$

In this model version, C gained by mycorrhizal fungi is only derived from plants, and C is only transferred out of soil through free-living microbial decomposition resulting in CO_2 release to the atmosphere. This is considered to be the null model, however, there is evidence mycorrhizal fungi use extracellular enzymes to decompose SOM similar to free-living decomposers in soil (Jones et al. 2012, Vaario et al. 2012). In the second model version ectomycorrhizal fungi (M) decompose SOM at a constant specific uptake rate (u_M) (Eq. 6).

$$V_2 = u_M M \quad \text{Eq. 6}$$

This function is used in place of V_i in the constant uptake model version. Third, we used the enzyme kinetics function applied to microbial activity to describe mycorrhizal activity. We assumed a maximum uptake rate independent of mycorrhizal biomass (Eq. 7).

$$V_3 = V_{\max,M} S / (S + K_M) \quad \text{Eq. 7}$$

This function is used in place of V_i in the variable uptake model version. Mycorrhizal enzyme activity is regulated by the availability of substrate (S) with half-saturation coefficient (K_M) where activity is 50% of maximum. The third model implicates that the mycorrhizal fungal specific uptake rate increases when mycorrhizal biomass decreases with decreasing NPP. We chose $V_{\max,M}$ and K values based on model simulations that produced realistic levels of mycorrhizal and soil C at equilibrium that are within a reasonable range of microbial parameter values (Table 1.1). We selected u_M , $V_{\max,M}$, and K_M parameter values such that models V_2 and V_3 would produce similar amounts of mycorrhizal biomass compared to V_1 , thus ensuring models V_2 and V_3 produce the same amount of soil carbon when plant productivity is 100%. We did this to have a common baseline against which to test changes in soil carbon in models V_2 and V_3 in response to reduction in plant productivity. While the simpler model V_2 may initially be more appealing than V_3 , model V_3 contains an indirect link between plant productivity (NPP) and mycorrhizal fungal activity as the substrate consumed is derived from plant inputs. Therefore, model V_3 is more dynamic and may be more relevant to natural systems.

Mycorrhizae grow with inputs from plants ($a_M P$) and soil (V_i) (Eq. 8).

$$dM / dt = e_1 a_M P + e_2 V_i - k_M M - r_M M \quad \text{Eq. 8}$$

We assume an energy tradeoff: ectomycorrhizae can gain C more efficiently from plants, since it is relatively labile, and less efficiently from soil ($e_1 > e_2$), since it is relatively recalcitrant. We consider the C that is lost as V_i is scaled down as mycorrhizal growth respiration. Mycorrhizal maintenance respiration (r_M) also accounts for C lost from the system, while mycorrhizal turnover (k_M) contributes C to soil. While mycorrhizal growth and functions feed back to net primary productivity (NPP), the feedback is implicit in our model. Since mycorrhizal turnover rate is much faster than the turnover rate for plants, mycorrhizal biomass reaches quasi-equilibrium as a function of plant

productivity. Thus, mycorrhizal feedback on NPP is equivalent to plant biomass feedback to itself, and reaches a steady state.

The free-living microbial community uses soil C (V_B) for respiration (r_B) and C is returned to soil through microbial mortality (k_B)

$$dB / dt = V_{\max,B} S / (S + K_B) - k_B B - r_B B \quad \text{Eq. 9}$$

Free-living microbial enzyme activity is assumed to have a maximum uptake rate ($V_{\max,B}$) regulated by the availability of substrate (S) with a half-saturation coefficient (K_B). We assume that the free-living microbial community uptakes carbon from soil following enzyme kinetic dynamics, a Michaelis-Menten function. Thus, free-living microbes have a variable uptake of C in all three models and mycorrhizae have a variable uptake only in model V_3 . In models V_2 and V_3 where mycorrhizae are decomposing, mycorrhizae and microbes are accessing the same soil C pool. This assumption is appropriate for the purposes of our model since we do not specify soil depth, even though we recognize that mycorrhizae and free-living microbes occupy spatially distinct niches defined by soil depth (Lindahl et al. 2007).

We made simplifying assumptions about the theoretical ecosystem in order to reduce model complexity. We are primarily interested in C cycling changes that occur on short ecological timescales, thus we only described pools that turn over rapidly. For example, because they are more labile we describe leaf litter and fine roots and not woody stems and coarse roots. While these assumptions limit the applicability of the model, they are appropriate for theoretical investigation of ectomycorrhizal activity responding to lower plant productivity. Since we made simplifying assumptions and have not attempted to produce a complex ecosystem model, we discuss results of models with mycorrhizal activity relative to one without mycorrhizal activity. This is appropriate for our questions given the theoretical nature of this work, but could be translated into absolute results for comparison with field observations after parameters are verified by experiments.

Parameterization and Model Simulations

Parameter values and ranges are mainly from previous studies on primary production, soil organic carbon degradation, and microbial physiology (references provided in Table 1). We estimated baseline parameter values based on steady-state mass balance. We assumed baseline net primary productivity (G) at $1000 \text{ g} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$, 50% of which passed to leaf litter fall, 30% of which passed to root litter, and the remains were allocated to mycorrhizae. With the baseline plant standing biomass assumed to be 10000 g C/m^2 from literatures, we estimated a_L , a_R , and a_M at 0.05, 0.03 and 0.02 respectively. Leaf litter was partially decomposed at soil surface, thus, 45% of NPP was respired before entering soil C pool ($450 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$). Mycorrhizal turnover and respiration rates were estimated to be 3.0 and 1.0 yr^{-1} , so that mycorrhizae function as a C sink in V_1 with $150 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ deposited in soil, and $50 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ is released as CO_2 . The rest of C fluxes out of the system by microbes at $500 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ in V_1 . The microbial turnover rate was estimated at 3.75 yr^{-1} , so that the carbon use efficiency was around 0.47 (Anderson and Domsch 1986, Six et al. 2006). The half-saturation coefficient was set at 5000 g C/m^2 to ensure the Michaelis-Menten kinetics were functional. There were no direct data available for estimation of maximum mycorrhizal C uptake, and half-saturation coefficient in V_3 . We assumed the maximum uptake rate of mycorrhizae to be around 1/8 of microbial uptake rate ($200 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ mycorrhizae; $1600 \text{ g C} \cdot \text{m}^{-2} \cdot \text{yr}^{-1}$ microbial). We compared two half-saturation coefficients for mycorrhizae, one assumed to be equal to microbial half-saturation ($K_M = 5000 \text{ g C/m}^2$). The other coefficient assumed faster uptake rate at low substrate concentration ($K_M = 2000 \text{ g C/m}^2$), which represents a trade-off between maximum uptake rate and substrate affinity. The parameter u in V_2 was adjusted to produce similar soil carbon storage between V_2 and V_3 at maximal plant productivity. Most estimated parameters and simulated C pools are within published ranges (Table 1.1).

We simulated C fluxes through plants, leaf litter, roots, ectomycorrhizal fungi, and soil. We solved the set of ordinary differential equations described above using the ode45 problem solver in MatLab (version R2011b, MathWorks, Natick, MA, USA). First, we used a latin hypercube sampling (LHS) algorithm (McKay et al. 1979) to develop

1000 sets of parameters values. The LHS algorithm allows an un-biased estimate of the average model output, with the advantage that it requires fewer samples than simple random sampling to achieve the same accuracy. Next, we conducted monte carlo (MC) simulations with the model using each of the 1000 sets of parameter under each of the 3 versions of the model, at monthly time steps until a steady state was reached (c. 1800 months). Monthly time steps were chosen based on the time scale of mycorrhizal turnover. Plant production was scaled randomly from 0% to 100% for each permutation. Only four permutations resulted in negative pool sizes and were excluded from model comparisons.

We compared changes of soil C storage at steady state in the constant uptake version (V_2) and the variable uptake version (V_3) as a percent of soil C storage predicted by the null model (V_1). We calculated C going to mycorrhizae from soil, which is zero in the null model, as a percentage of total C flux from plants and soil to mycorrhizae. Soil C is taken up by microbes and mycorrhizae, and we calculated the percentage of C flux from soil to mycorrhizae as a percent of total flux out of soil to mycorrhizae and microbes. In the null model, mycorrhizal C was stored in soil after maintenance respiration was deducted. When mycorrhizal decomposition was accounted for, we calculated net flux of C from mycorrhizae to soil.

We studied global sensitivity of parameters to soil carbon pool, using the estimated baseline parameters. Based on the MC ensemble simulations results above, we calculated a parameter sensitivity index using partial rank correlation coefficient (PRCC). PRCC is a robust sensitivity measure for nonlinear but monotonic relationships between parameters and model outputs (Marino et al. 2008), which performs a partial correlation between specific parameter and model outputs on rank-transformed data taking into account the remaining parameters.

Results

Declines in plant productivity reduced soil C storage across all strategies (V_1 , V_2 , V_3). When ECM function as decomposers (V_2 and V_3) their effect on soil C dynamics varied with plant productivity (Fig. 1.2, data shown relative to null model V_1). At zero

productivity there are no fresh inputs of C to the system. However, soil, mycorrhizal, and microbial C pools remain positive at steady state. This is because of a tight recycling of C that is taken up by mycorrhizae and microbes. Their necromass and waste products then regenerate soil organic matter, which may contribute to soil carbon buildup (Cotrufo et al 2013). The effect of the two ECM decomposition strategies diverged as plant productivity was reduced. ECM with constant uptake rates (i.e., V_2) had less of an effect on soil C storage than ECM with variable uptake rates (i.e., V_3). Changes in mycorrhizal biomass were likely the mechanism for the difference between the two models. Less mycorrhizal biomass means lower total enzymes are exuded when mycorrhizal activity is modeled as constant. However, relatively more enzymes are exuded when mycorrhizal decomposition is modeled as variable with substrate availability.

In the variable uptake model (V_3) the level of plant productivity altered the amount of C taken up from soil by mycorrhizae. Mycorrhizae acquired C from two sources: plants and soil. The percent of C acquired by mycorrhizae from soil out of total C from soil and plants did not vary with plant productivity in the constant uptake model. The percent of C acquired by mycorrhizae from soil gradually increased in the variable uptake model (Fig. 1.3).

Mycorrhizae and the free-living soil microbial community simultaneously decomposed soil C and their combined activity decreased soil C storage in models V_2 and V_3 . At the highest plant-productivity levels a small amount of C moving from soil to biotic pools went into mycorrhizal biomass rather than free-living microbial biomass (Fig. 1.4). This C pathway remained at the same level in the constant uptake model (*data not shown*). In the variable uptake model the amount of C moving from soil to mycorrhizae gradually increased. Thus, even at low plant productivity, free-living microbes acquired most of decomposable soil C. In addition to decomposing and assimilating C in soil, mycorrhizae contribute to C storage as their tissues decay. The net amount of C transferred from decaying mycorrhizal biomass to soil was similar for the constant and variable models when plant productivity was high (Fig. 1.5). However, when plant productivity was reduced, the effect of mycorrhizae on soil in the constant model

deviated from the variable model. The net amount of C transferred to soil by mycorrhizae was greater under the constant uptake model compared to the variable uptake model. At low levels of plant productivity, the effect on soil storage became negative, meaning there was net loss of C from the soil to mycorrhizae.

Using soil C pool size as the response variable, our sensitivity analysis showed parameters related to microbial activity are important across all three models (Fig. 1.6). This is likely because microbes directly regulate decomposition. Soil C was also affected by plant C allocation patterns and soil respiration. The more C transferred to soil from plant and mycorrhizal litter, the higher soil respiration. Parameters related to mycorrhizal activity showed different sensitivities among the three models. In model V_1 , mycorrhizae are a source for soil C similar to plant litter. But plant C allocation to mycorrhizal biomass (a_M) and mycorrhizal respiration (r_M) are less important than plant litter in controlling soil C storage. On the other hand, model V_2 predicts a strong mycorrhizal uptake of C from soil and soil C therefore has a linear relationship to mycorrhizal biomass. Thus, a_M and u_M are important parameters that are negatively related to the soil C pool. The sensitivity index of mycorrhizal respiration (r_M) is positive, suggesting that soil carbon reductions in model V_2 are due to carbon stored in mycorrhizal biomass not respired to the atmosphere. Most parameters in model V_3 have sensitivity similar to model V_1 , with a few exceptions unique to model V_3 . The soil C pool in V_3 is not sensitive to plant allocation to mycorrhizal biomass, which means that mycorrhizae can adjust C uptake from soil depending on how much C they are allocated from plants.

Discussion

Biotic interactions that drive ecosystem functions such as decomposition and mycorrhizal-plant interactions contribute significantly to ecosystem functioning (van der Heijden et al. 1998). Yet, ecologists have only begun to consider how ectomycorrhizal utilization of plant assimilated C can affect soil C storage within a predictive framework (Orwin et al. 2011). To our knowledge this model is the first to incorporate decomposition of organic matter and subsequent C acquisition by ectomycorrhizal fungi.

The work here leads to two primary conclusions: ectomycorrhizal fungi have a substantial effect on soil C storage, and the effect of ectomycorrhizae on C storage is markedly different depending on plant productivity, at least in the variable uptake model. We recognize that numerous biotic interactions within an ecosystem contribute to soil C storage. However, increasing the number of interactions in a model will not necessarily generate more reliable model predictions. Model efforts should focus on those interactions that have the capacity to significantly change model predictions. Plant-mycorrhizae-soil interactions play a pivotal role in ecosystem C dynamics and including them in models is both important and feasible.

Mycorrhizal activity alters soil C dynamics

The two strategies we modeled for mycorrhizal C uptake affected soil C storage differently. Soil C storage was reduced more when specific enzyme activity was variable with plant productivity and soil substrate availability compared to when mycorrhizal uptake was constant. The two models converged in their predictions of soil C storage at high levels of plant productivity; thus, in simulated ecosystems with high plant productivity the mechanism for mycorrhizal C acquisition from soil does not influence soil C storage differently than a simulated ecosystem lacking mycorrhizal activity.

Regardless of strategy, soil C storage was affected by mycorrhizae because they were an additional decomposer group. As plant production declined, fungal biomass declined, contributing to the relatively small effect of mycorrhizae on soil C storage at low plant productivity. In our model, productivity affects the absolute and relative amount of C transferred from plant to mycorrhizal fungi because the allocation parameter a_M is constant. Fertilization can shift allocation patterns, thus changing the relative amount of C transferred from plants to mycorrhizal fungi, which may affect mycorrhizal activity in the same way as reduced total productivity as modeled here. In this respect, our result corroborates several other studies where N fertilization reduces relative C allocation to mycorrhizae and leads to fewer mycorrhizal root tips and lower mycorrhizal production (Pritchard et al. 2014), mycorrhizal respiration, and sporocarp production (Hasselquist et al. 2012). Still, the mechanism remains unclear and we

suggest experiments manipulate plant productivity or relative allocation patterns and measure ectomycorrhizal fungal specific enzyme activity *in situ*, or at least measure potential activity in a controlled setting in combination with ectomycorrhizal fungi biomass. Activity of plant cell wall-degrading enzymes in the mycorrhizosphere increases when relative allocation of C to mycorrhizae is reduced via nitrogen fertilization (Jones et al. 2012), but it is unclear whether this effect is directly attributable to mycorrhizal activity. While we are unaware of methods for measuring enzyme activity rates *in situ*, we think proteomic or metabolomic methods may be a useful tool to exploit. Others have used proteomic methods to suggest mycorrhizal genera rather than other microbial groups produce soil enzymes (Rineau et al. 2012). If we could target general ECM enzyme activity compared to free-living microbial activity, we could produce a validated range of expected mycorrhizal enzyme activity rates that would strengthen model predictions.

Plant productivity affected mycorrhizal C fluxes in the variable uptake model but not in the constant uptake model. In the variable uptake model, mycorrhizae acquired more C from soil than plants, simultaneously reducing the role of the free-living microbial community in decomposition. Mycorrhizae acquired more C from soil in a low productivity ecosystem compared to a highly productive ecosystem. The amount of C acquired by mycorrhizae was constrained by mycorrhizal C use efficiency, a parameter that remains to be experimentally evaluated. If ectomycorrhizae are efficient at using soil C, our model underestimated the amount of soil organic matter decomposed by mycorrhizae. Plant productivity was a key driver of mycorrhizal activity in the variable uptake model, but not in the constant uptake model. The constant uptake model assumed mycorrhizae acquire C as a secondary product while they mine organic matter for nutrients (Talbot et al. 2008). Plant productivity therefore alters the absolute amount of soil C decomposed by mycorrhizae because mycorrhizal biomass declines. However, the relative amount of soil C decomposed by mycorrhizae compared to the free-living microbial community does not change. To disentangle the effects of mycorrhizal from the effects of the free-living microbial community on soil C decomposition, future studies that manipulate plant productivity should measure mycorrhizal and free-living microbial

biomass and partition decomposition measurements (litter mass loss, soil C turnover, soil C respiration) between the mycorrhizal and free-living pools. Although the pattern between ectomycorrhizal fungal enzyme activity and plant production was striking, the quantitative effects of ectomycorrhizal fungal enzyme activity on soil C are contingent upon experimental validation of mycorrhizal enzyme kinetics. Tedersoo and colleagues (2012) measured ectomycorrhizal enzyme activity in a tropical rainforest, but studies across different ecotypes are needed to produce a broadly applicable range of parameters.

Recommendations for improving future decomposition models

Our work demonstrates that incorporating microbial functional groups into soil decomposition models may improve our understating of soil C dynamics - especially when plant productivity varies. Plant productivity can alter soil C storage via altering the activity of the soil microbial community. Soil respiration increases when plant productivity is reduced through defoliation and this increase mirrors increases in enzyme activity within root tips, which may be indicate mycorrhizal activity (Cullings et al. 2008, Cullings and Hanely 2010). The amount of soil C mineralized by mycorrhizae could be quantified using stable C isotope techniques (Heinemeyer et al. 2006, Heinemeyer et al. 2007). Increases in extracellular enzyme activity could also result from increased free-living microbial abundance feeding on decaying mycorrhizal tissue (Lindahl et al. 2010). While we have not explored this phenomenon here, we note that some types of mycorrhizae may enhance decomposition by increasing the activity of the free-living soil microbial community (Hodge et al. 2001). Still, field measurements that directly target the rate of soil C uptake and mineralization by ectomycorrhizal fungi are needed to improve our model predictions.

The flux of soil C to mycorrhizae compared to the total C flux from plants and soil to mycorrhizae seemed high relative to other empirical studies. Treseder et al. (2006) found 2% of C in mycorrhizal biomass was derived from recently decomposed leaf litter, suggesting mycorrhizae are responsible for decomposing a negligible fraction of soil C. However, this result can be interpreted in at least two ways. First, it may be a product of mycorrhizal C use efficiency. We have assumed that mycorrhizae are less efficient at

assimilating soil-derived C due to soil C physicochemical complexity compared to photosynthate allocated to mycorrhizae. Treseder et al. (2006) did not measure the amount of soil C respired by mycorrhizae. It is possible that mycorrhizae break down a substantial amount of soil C but assimilate little of it into biomass and instead release the majority as CO₂. Field studies that isolate mycorrhizae and use isotopic tracers to track soil C into mycorrhizal biomass and respiration and laboratory C use efficiency studies are needed to confirm this idea. Secondly, the Treseder et al. (2006) result may indicate activity specific to mycorrhizal root tips rather than all mycorrhizal fungal tissues. Mycorrhizal tissues vary in expression of genes encoding different aspects of decomposition, such as enzyme production, nutrient assimilation, and transport (Wright et al. 2005). It may be that Treseder et al. (2006) targeted tissues not involved in the ecosystem processes they measured. We suggest testing the hypothesis that foraging hyphae exude a greater abundance or diversity of extracellular enzymes and assimilate more soil-derived C than tissues associating directly with plant roots by measuring enzyme activity at various distances away from roots.

The model we developed considers additional features that are significant to C cycling by adding an additional decomposer group with the flexibility to change its decomposition rate based on environmental change. Widely used C cycle models produce very different results for C pool estimates (Todd-Brown et al. 2012). Adding biological details such as microbial community changes, especially among functional groups, in response to nutrient availability and partitioning fluxes of C among roots and mycorrhizae could result in improved model predictions (Chapin et al. 2009, Todd-Brown et al. 2012, Treseder et al. 2012).

Many models have one or many soil compartments that turnover at constant rates and enzyme activity by microbes is assumed to be uniform across decomposer groups, but this is not an accurate representation of natural ecosystems (Sinsabaugh 1994, Hanson et al. 2008). Soil C dynamics are partly determined by microbial traits (Allison 2012), guilds that partition resource niches (de Boer 2005, Moorhead and Sinsabaugh 2006), and microbial community structure with many functional groups (Orwin et al. 2011). Adding microbial enzymatic decomposition to C models resulted in

increased prediction accuracy compared to field-measured responses (Lawrence et al. 2009). To our knowledge, only a few other models include mycorrhizal fungi as a unique guild (Deckmyn et al. 2008, Meyer et al. 2010, Orwin et al. 2011). In these, mycorrhizal fungi have the ability to take up nutrients from soil, exchange these for C from plants, and subsequently alter soil C storage. Mycorrhizal biomass, nutrient uptake rates (Meyer et al. 2010, Orwin et al. 2011), and C allocation from plants to mycorrhizae (Orwin et al. 2011) are important mycorrhizal parameters in simulated C dynamics. However, previous models lack the function of mycorrhizae to directly contribute to decomposition of soil C. Our study includes direct effects of mycorrhizae on soil C and indicates that ectomycorrhizal fungi can alter soil C storage through decomposition activity suggesting that pathway should be included in models.

Conclusions

Using mathematical theory, we demonstrate that biotic interactions can affect predictions of ecosystem functions. While biotic interactions are often complex, we developed a simple function to model the mycorrhizal switch in function from plant symbiont to decomposer. Mechanisms causing a switch need to be further parameterized using experiments before our model can confidently predict accurate fluxes and pools of C in an ecosystem. We suggest that mycorrhizal biomass, specific enzyme activity, and respiration be measured in decomposition experiments that manipulate plant productivity in order to increase the utility of this model. Other rhizosphere interactions could be modeled by manipulating external factors (e.g., plant productivity) and internal regulators (e.g., carbon use efficiency), which may depend upon the biomass of an interacting species.

Experiments and models also need to explore when and where ECM decompose soil organic matter. Given C allocation from plants to ECM varies with season, there is likely seasonal variation in when ECM decompose soil organic matter. ECM growth and enzyme activity is highest in the autumn (Wallander et al. 2001, Buee et al. 2005, Kaiser et al. 2010), when total C received from plants is low because of reduced photosynthesis. Similarly, mycorrhizae may access soil C in the spring during bud break

when C allocation to belowground structures is low (Courty et al. 2007). Thus, decomposition by ECM could be highest in early spring and autumn. Soil organic matter decomposition by ECM may be higher in areas where nitrogen is limiting to plant and microbial growth. While ECM decomposition may be possible in nutrient-limiting ecosystems, it may not be the prevailing process that regulates C stocks in soil. For example, in N-limited and ECM dominated boreal systems C stocks are higher than in N-rich systems dominated by arbuscular mycorrhizal fungi (Averill et al. 2014), possibly owing to nutrient competition between plants and fungi. In order to acquire nitrogen for uptake and growth, the microbial community must mineralize C, thus C and nitrogen processes in soils are tightly coupled. When investigating how mycorrhizal fungi alter soil organic matter decomposition, we suggest that experimentalists target nitrogen-limited ecosystems during the fall and the spring. We also think temporal dynamics as well as the nutrient status of an ecosystem should be incorporated into modeling efforts. Mycorrhizal fungi clearly have the capacity to decompose organic matter and our theoretical model demonstrates they can be important in regulating processes pertinent to C cycling. Thus, we conclude that incorporating complex ecological interactions into C cycle models is feasible and could adjust model predictions significantly.

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Appendix: Figures

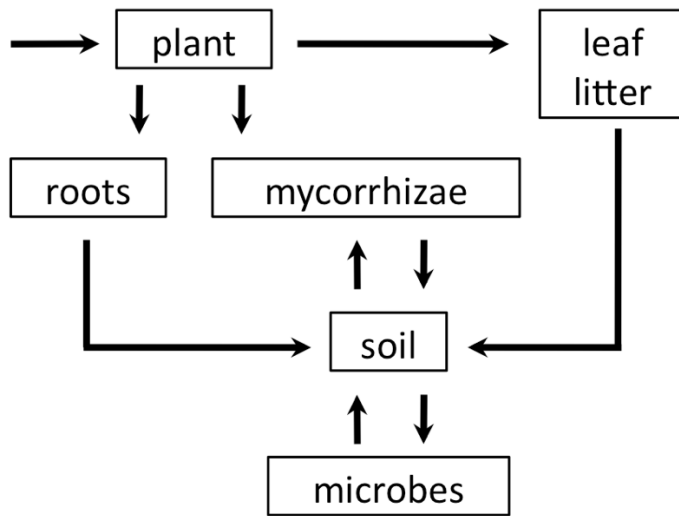


Figure 1.1. Conceptual diagram of belowground fluxes and pools of carbon. Carbon fluxes (arrows) between pools (boxes) where it is stored in plant tissues, mycorrhizae, free-living microbial biomass and labile soil organic matter. Equations developed for the conceptual system are given in the Model Description.

Figure 1.2. Changes in soil carbon storage in two alternative models. Increasing plant productivity (parameter s) in the constant uptake model version (dashed line) and the variable uptake model version (solid line) is shown as a percentage of soil carbon storage predicted by the null model. Mycorrhizal fungal activity was simulated using parameters similar to microbial activity (top panel) and with a faster uptake rate (bottom panel; see Parameterization and Model Simulations for details).

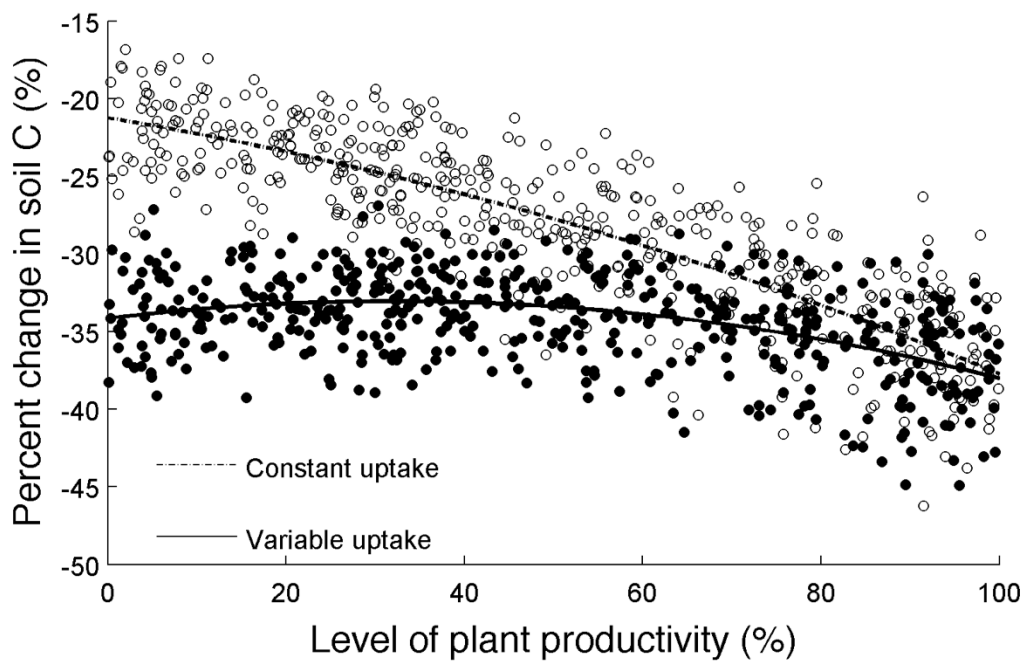
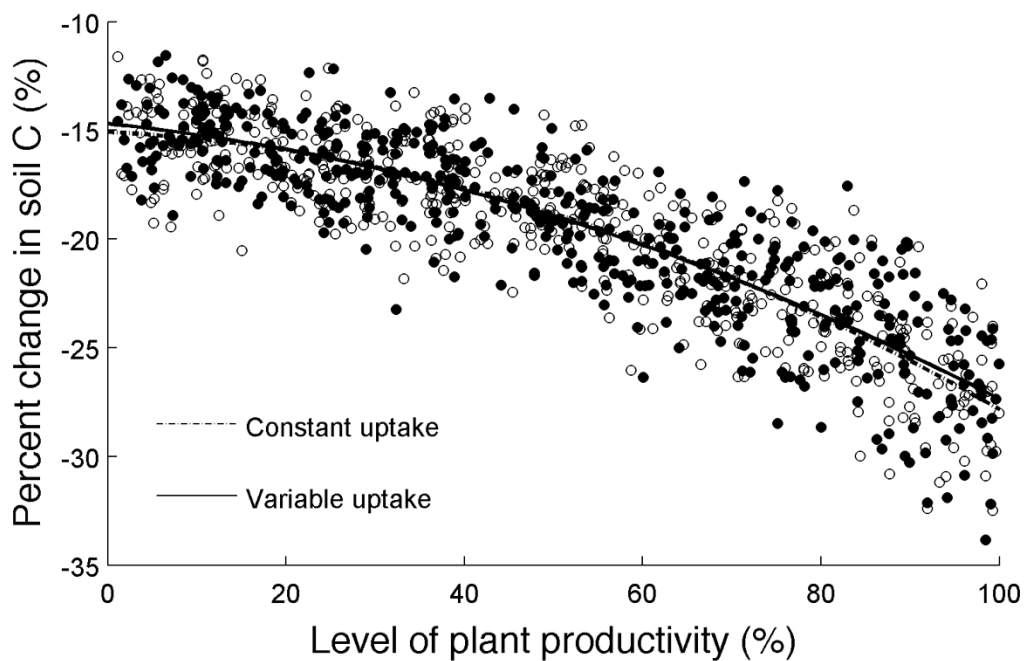


Figure 1.2. Continued.

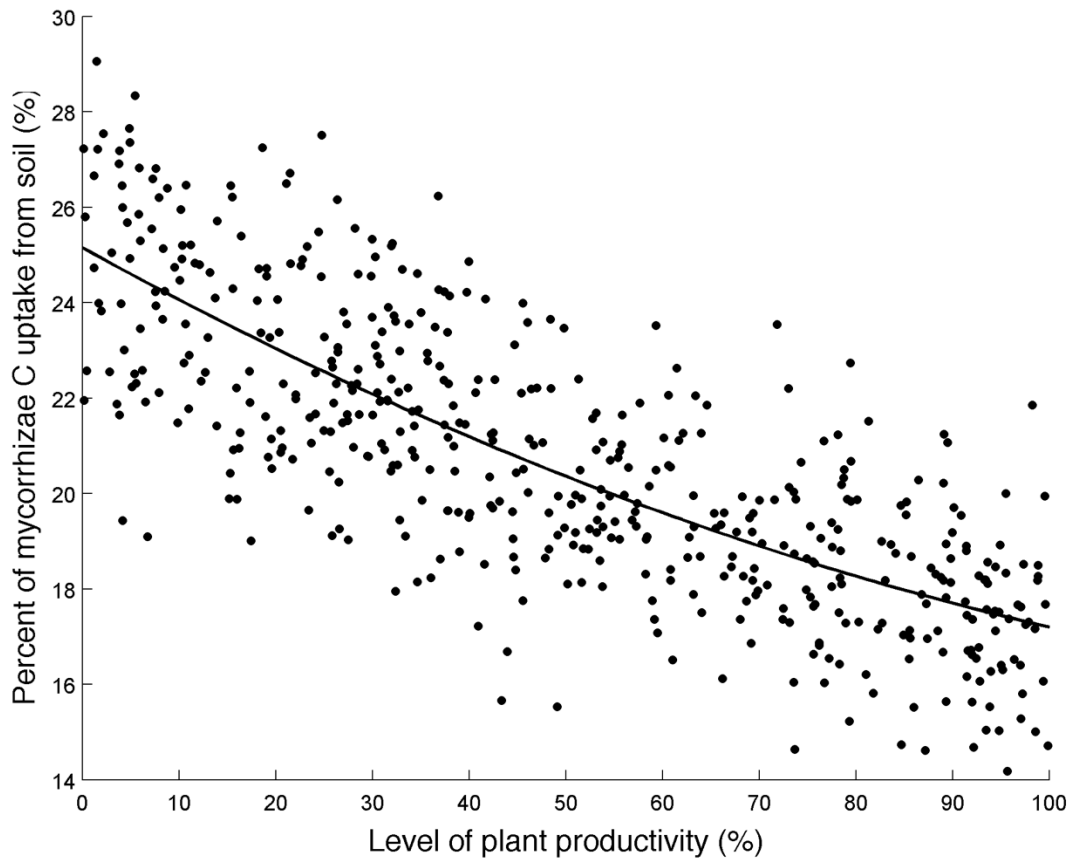


Figure 1.3. Mycorrhizal carbon uptake. Carbon flux to mycorrhizae from soil as a percent of total fluxes coming from soil and plants declines with plant productivity (parameter s) in the variable uptake model version.

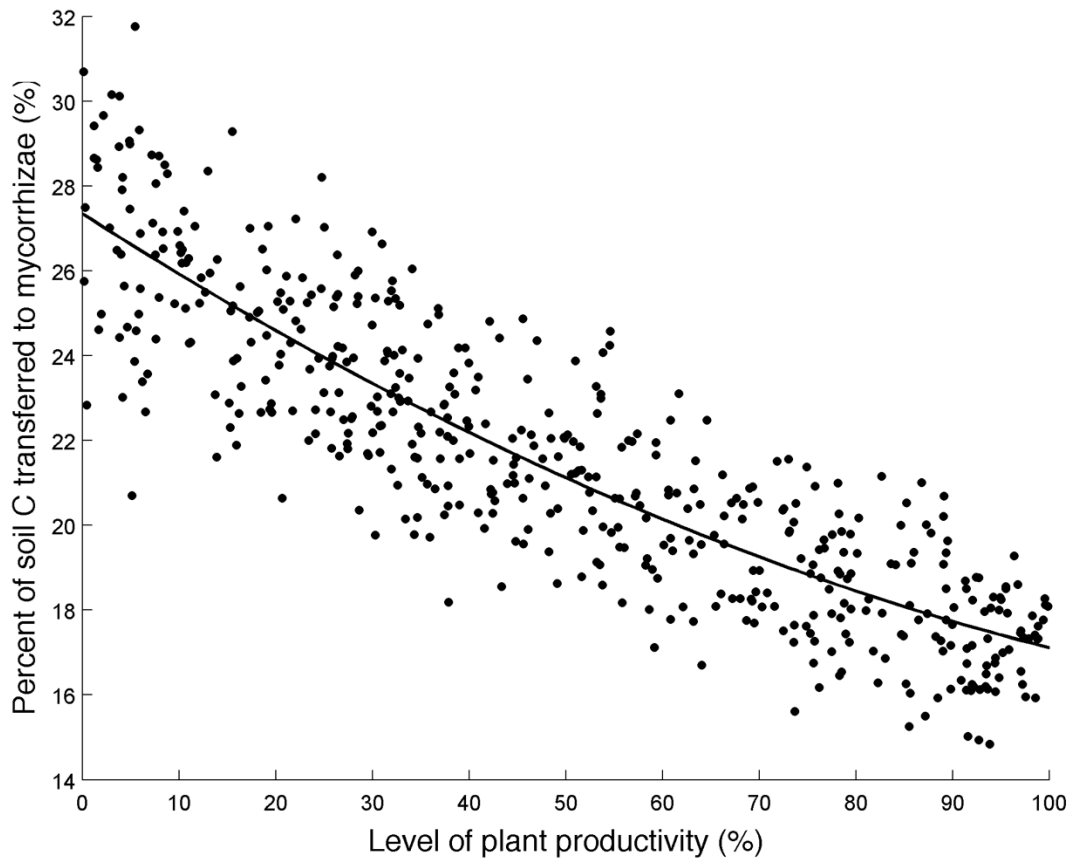


Figure 1.4. Soil carbon transferred to mycorrhizae. Carbon flux to mycorrhizae from soil as a percent of total fluxes from soil to mycorrhizae and free-living microbes declines with plant productivity (parameter s) in the variable uptake model version.

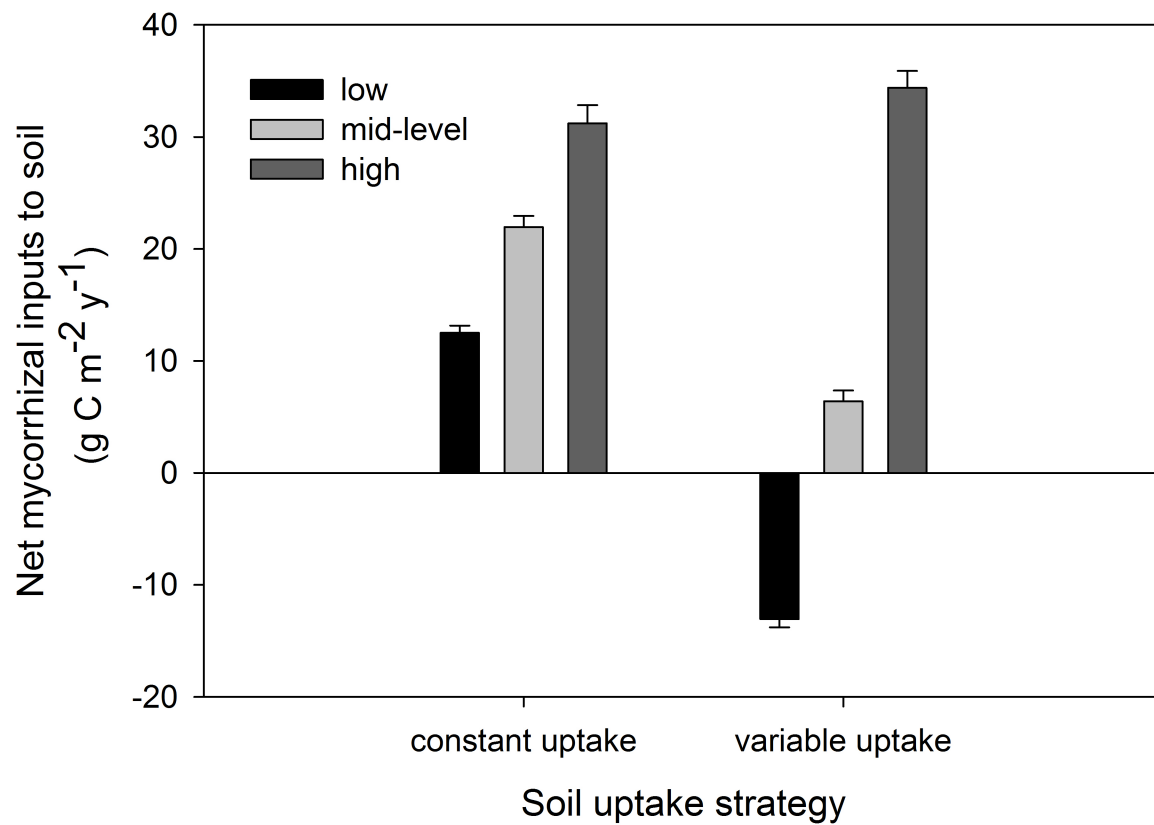


Figure 1.5. Net flux of carbon from mycorrhizae to soil. After mycorrhizal decomposition is accounted for, the constant and variable uptake model versions vary in their flux of carbon from mycorrhizae to soil across three levels of plant productivity: low (40%), mid-level (70%), and high (100%). Bars show mean and SE of 500 permutations with parameters varied 10%.

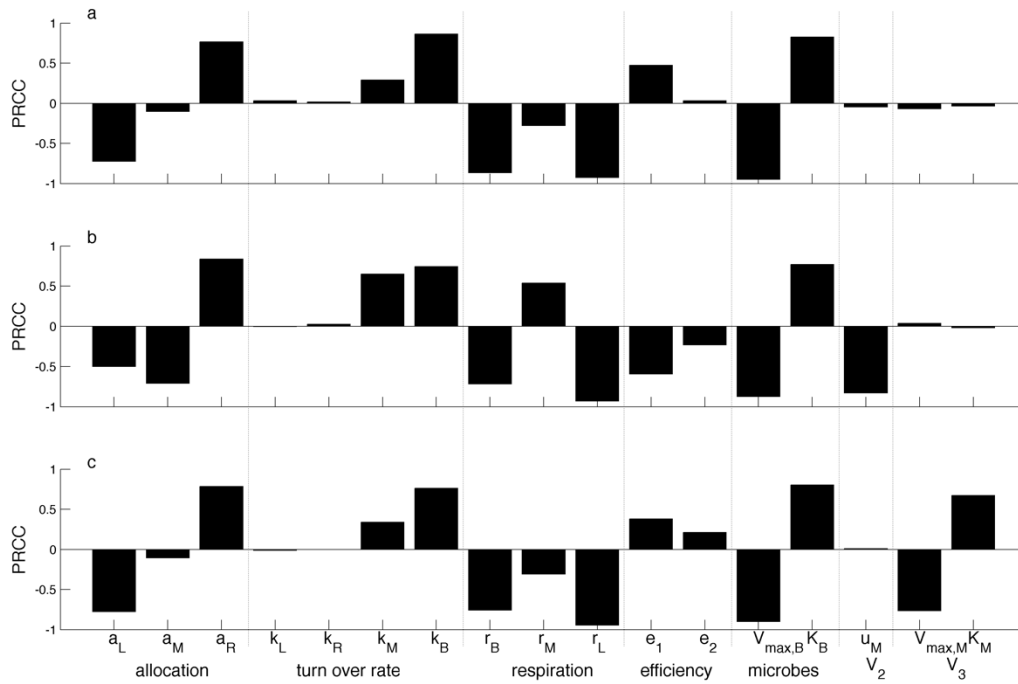


Figure 1.6. Analysis of sensitivity of soil carbon pool to parameters. For (a) model V_1 , (b) model V_2 , and (c) model V_3 , we varied parameters by 10% and show the strength of that parameter's effect on predicted soil C (partial ranked correlation coefficient; PRCC). A higher absolute PRCC indicates that soil C predictions are sensitive to that parameter, and strong empirical support of that parameter is needed to produce reliable quantitative model predictions. Parameters are separated by vertical dash lines as categories of allocation, turn over rate, respiration, efficiency, microbes, and parameters unique to model V_2 and V_3 .

Appendix: Tables

Table 1.1. Description of parameters used in the model. Sources provide a close approximation to the parameters.

Parameter	Description	Value	Range	Units	Reference
G	net primary productivity	1000.00	800 – 1300.000	$\text{g C} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$	Whittaker and Likens 1973
a_L	leaf litter allocation	0.05	0.014 – 0.100	y^{-1}	Litton et al. 2007
k_L	litter turnover	0.50	0.032 – 0.624	y^{-1}	Gholz et al. 2001
r_L	fraction of litter respired	0.90		-	
k_R	root turnover	0.30	0.036 – 0.501	y^{-1}	Gholz et al. 2001
a_M	mycorrhizal allocation	0.02	0.008 – 0.060	y^{-1}	Hobbie 2006
r_M	mycorrhizal respiration	1.00	0.400 – 1.500	y^{-1}	Fenn et al. 2010
a_R	root allocation	0.03	0.120 – 0.070	y^{-1}	Raich and Nadelhoffer 1989
k_M	mycorrhizal turnover	3.00	1.740 – 5.210	y^{-1}	Rygiewicz et al. 1997, Treseder et al. 2006, McCormack et al. 2010, Orwin et al. 2011
k_B	microbial turnover	3.75	0.439 – 11.388	y^{-1}	Wang et al. 2012
K_M	mycorrhizal half-saturation	5000.00		g C/m^2	
K_B	microbial half-saturation	5000.00		g C/m^2	Wang et al. 2012
$V_{\max,M}$	maximum mycorrhizal C uptake	200.00		$\text{g C} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$	

Table 1.1. Continued.

Parameter	Description	Value	Range	Units	Reference
$V_{max,B}$	maximum microbial C uptake	1600.00	400 – 5800.00	$\text{g C} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$	Wang et al. 2012
e_P	plant-acquisition efficiency	1.00		-	
e_S	soil-acquisition efficiency	0.30		-	
s	scales G	0 – 1.00		-	
u	specific mycorrhizal C uptake	2.50		$\text{g C} \cdot \text{g}^{-1} \text{ C of } M \cdot \text{y}^{-1}$	
r_B	microbial respiration	5.00	1.75 – 7.00		Hogberg et al. 2001

Chapter 2: Interactions among roots, mycorrhizae, and free-living microbial communities differentially impact soil carbon processes

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Abstract

Plant roots, their associated microbial community, and free-living soil microbes interact to regulate the movement of carbon from the soil to the atmosphere, one of the most important and least understood fluxes of terrestrial carbon. Our inadequate understanding of how plant-microbial interactions alter soil carbon decomposition may lead to poor model predictions of terrestrial carbon feedbacks to the atmosphere. Roots, mycorrhizal fungi, and free-living soil microbes can alter soil carbon decomposition through exudation of carbon into soil. Exudates of simple carbon compounds can increase microbial activity because microbes are typically carbon-limited. When both roots and mycorrhizal fungi are present in the soil, they may additively increase carbon decomposition. However, when mycorrhizae are isolated from roots they may limit soil carbon decomposition by competing with free-living decomposers for resources. We manipulated the access of roots and mycorrhizal fungi to soil *in situ* in a temperate mixed deciduous forest. We added ^{13}C -labelled substrate to trace metabolized carbon into respiration, measured carbon-degrading microbial extracellular enzyme activity, and soil carbon pools. We used our data in a mechanistic soil carbon decomposition model to simulate and compare the effects of root and mycorrhizal fungal presence on soil carbon dynamics over longer time periods.

Contrary to what we predicted, root and mycorrhizal biomass did not interact to additively increase microbial activity and soil carbon degradation. The metabolism of ^{13}C labeled starch was highest when root biomass was high and mycorrhizal biomass was low. These results suggest that mycorrhizae may negatively interact with the free-living microbial community to influence soil carbon dynamics, a hypothesis supported by our enzyme results. Our steady-state model simulations suggested that root presence increased mineral-associated and particulate organic carbon pools, while mycorrhizal fungal presence had a greater influence on particulate than mineral-associated organic carbon pools. Our results suggest that the activity of enzymes involved in organic matter

decomposition was contingent upon root-mycorrhizal-microbial interactions. By using our experimental data in a decomposition simulation model, we show that root-mycorrhizal-microbial interactions may have longer-term legacy effects on soil carbon sequestration. Overall, our study suggests that roots stimulate microbial activity in the short-term but contribute to soil carbon storage over longer periods of time.

Introduction

Ecological interactions in above-ground communities shape ecosystem processes such as net primary productivity (Hooper *et al.* 2005); however, less is known about how interactions below-ground affect the structure and function of ecosystems (Hogberg & Read 2006; van der Heijden *et al.* 2008; Classen *et al.* 2015). Below-ground soil microbial communities decompose soil organic matter and their degrading activity results in carbon loss to the atmosphere (Fenn *et al.* 2010). Because soils are the largest terrestrial carbon pool, a change in soil microbial carbon decomposition rates could influence the global carbon budget and climate-carbon feedbacks. The uncertainty in soil community interactions contributes to the uncertainty in global models that currently do not explicitly include soil microbial communities (Todd-Brown *et al.* 2012; Treseder *et al.* 2012). Free-living soil bacteria and fungi (hereafter, microbes) are key players in decomposition and thus terrestrial carbon-cycling processes. Microbes secrete extracellular enzymes that target specific compounds in soil organic matter in order to gain carbon to build microbial biomass (Nannipieri *et al.* 2002). After breaking apart organic matter the microbial community mineralizes it, which provides plants with nutrients that are essential for them to grow. This degradation and mineralization process releases large amounts of carbon from the soil back to the atmosphere as CO₂ (Fenn *et al.* 2010). Microbial communities are integral to decomposition and carbon release, yet we still have a poor understanding of what drives microbial processes (Bardgett & van der Putten 2014).

Plant roots interact with the soil community in a diversity of ways (Hogberg *et al.* 2007; Phillips *et al.* 2012; Clemmensen *et al.* 2013). For example, roots and mycorrhizae can exude carbon substrates into the soil, which affects microbial communities and ecosystem functions (Bais *et al.* 2006; Phillips 2007; de Graaff *et al.* 2010). If given a source of easily degraded carbon, such as root exudates, microbial communities will increase decomposition of old (i.e., >10 y) organic matter (Kuzyakov *et al.* 2010) and mineralize nutrients that plants can take up. This process, described as priming, may occur when roots release polysaccharides (Jaeger *et al.* 1999) that microbes can easily use as an energy source. Labile carbon sources such as

polysaccharides provide microbes with energy to gain carbon by decomposing more chemically complex organic matter (Kuzyakov 2010; Paterson & Sim 2013). In a laboratory study, soil bacteria and fungi increased their metabolic activity stimulating decomposition of soil-derived ("old") and plant-derived ("new") carbon and respiration rates at low simulated root exudation levels (de Graaff *et al.* 2010). However, the pattern changed at high levels of root exudation, where decomposition rates were reduced by 50% (de Graaff *et al.* 2010). Competition for other resources among microbes may increase when carbon limitation is alleviated, thus interactions among the microbial community may explain these counterintuitive patterns (Fontaine *et al.* 2003).

Plant roots and mycorrhizal fungi interact in different ways and the outcome of their interactions in turn affects microbial activity and related decomposition processes (Moore *et al.* 2015). Plant roots and mycorrhizal fungi are tightly linked in an association that can be symbiotic or parasitic (Johnson, Graham, & Smith 1997) and mycorrhizae can facilitate or compete with other soil microbes (Frey-Klett *et al.* 2011). Mycorrhizal fungi can facilitate the free-living microbial community by releasing organic compounds, which stimulate microbial growth more than non-mycorrhizal roots (Barea *et al.* 2005). In turn, microbes, specifically those identified as mycorrhizal-helper bacteria, can facilitate mycorrhizae by stimulating mycorrhizal colonization of plant roots as well as hyphal development (Garbaye 1994). Mycorrhizal fungi can enhance the decomposition of organic matter by hyphosphere microbes (Moore *et al.* 2015) and subsequently outcompete those microbes for nutrients such as nitrogen (Hodge, Campbell, & Fitter 2001) and phosphorus (Brooks *et al.* 2011). Mycorrhizal fungi can also suppress microbial activity to better compete for soil nutrients (Lindahl *et al.* 1999) thereby slowing decomposition (Gadgil & Gadgil 1975). Competition between roots, mycorrhizal fungi, and free-living microbes for soil nitrogen can lead to increased soil carbon stocks as microbial activity is suppressed (Averill, Turner, & Finzi 2014). Interactions among roots, mycorrhizae, and the free-living microbial community are clearly complex.

There is a general consensus in the literature that roots stimulate microbial activity, but how root-mycorrhizal-microbial interactions alter soil carbon decomposition rates is less clear (Phillips, Brzostek, & Midgley 2013). Our project aimed to disentangle

some of the uncertainty around these complex interactions. We designed *in situ* soil mesocosms that reduced root and mycorrhizal biomass and thus created a gradient of root and mycorrhizal influence on soil processes. We took advantage of this gradient to explore how microbial activities and soil carbon pools changed when parts of the soil community were reduced or removed. Because root-microbe interactions are driven by soil nutrient availability, and given our study was conducted in a temperate forest likely limited by soil nitrogen, we anticipated roots and mycorrhizal fungi would stimulate microbial activity. Specifically, we predicted that when root and mycorrhizal fungal biomass was high, microbial activity and decomposition rates would also be high, resulting in greater soil respiration and processing of “old” soil organic matter. Similarly, when biomass of the two groups was low, we predicted activity and respiration would also be relatively low. We experimentally manipulated the soil communities *in situ* in a mixed deciduous temperate forest and used a ^{13}C labeled substrate as a tracer to track the source of carbon respired by the soil microbial community. To explore if the presence of roots and mycorrhizal fungi had a larger impact on longer-term decomposition patterns that extended beyond the length of our field experiment, we used our experimental results in a simulation model. We predicted that interactions between roots, mycorrhizal fungi, and the free-living microbial community would affect processes in an additive way leading to changes in the soil carbon pool and that the effects of these shorter-term interactions would emerge in a decomposition model that extrapolates processes over 30 years.

Materials and methods

We conducted our experiment at the Walker Branch Watershed (*lat*: 35.957889, *long*: -84.286692) in Oak Ridge, TN USA. The site was located in a 20 m × 20 m area on a ridge where the aspect was flat. Our plots were located in a closed-canopy deciduous forest dominated by ectomycorrhizal and arbuscular mycorrhizal trees including hickory (*Carya spp.*), oak (*Quercus spp.*), and maple (*Acer spp.*). The dominant understorey species were muscadine (*Vitis rotundifolia*) and poison ivy (*Toxicodendron radicans*). Mean annual temperature was 14.5°C and mean annual precipitation was 1400 mm yr⁻¹.

¹. Bulk density of the B-horizon was 1.4 to 1.5 g cm⁻³ with a pH between 4 and 5 (Jardine *et al.* 2006). Soils at the site are classified as Ultisols of the Fullerton-Pailo complex (USDA Natural Resources Conservation Service Soils).

Experimental design and analysis

To test the effect of roots and mycorrhizal fungi on microbial activity and soil properties related to carbon dynamics, we crossed root and/or mycorrhizal exclusion treatments with a ¹³C labeled starch addition. We constructed 30 soil mesocosms from PVC pipe (15 cm long, 5 cm diameter) (Phillips *et al.* 2012). In each of the PVC mesocosms, we cut two 10.5 × 8 cm rectangular openings into the sides and covered these openings and the circular bottom of the PVC mesocosm with 1.45 mm, 55 μm, or 5 μm stainless steel mesh to allow root and mycorrhizal access, exclude roots, or exclude roots and mycorrhizae, respectively. We were unable to measure effects of roots without mycorrhizal fungi (a hypothetical treatment) because the methods used to remove mycorrhizal fungi (e.g., fungicide) would alter the microbial community, thereby reducing our experimental inference. The mesh was affixed to each mesocosm using Household Goop adhesive (Eclectic, Eugene, OR USA).

We installed the mesocosms on May 15, 2013 at the beginning of the growing season. Mesocosm sites were selected randomly throughout the forest understorey with a minimum spacing among mesocosms of 0.5 m. Each mesocosm was filled with soil removed (5 × 15 cm hammer corer, AMS, Inc., American Falls, ID) from the exact location in which the mesocosm was inserted. Roots were removed from the soil and the soil was mixed with inert sand (1:1 v/v) to promote drainage in this high-clay soil. Prior to establishing the experiment, we tested several ratios of soil-sand mixtures and determined a 1:1 ratio reduced water from pooling on the soil surface. Approximately 350 g of the soil-sand mixture was returned to the mesocosm and each mesocosm was returned to the site it was collected from. Mesocosms were placed vertically in the soil profile so that the mesh-covered openings were completely below the soil surface and there was a tight seal with the soil profile. Within each mesocosm, we buried a 5 cm by 3.5 cm, 50 μm nylon mesh mycorrhizal in-growth bag filled with approximately 30 g

autoclaved quartz sand approximately 5 cm below the soil surface. Mesocosms stabilized in the field for 16 weeks to allow root and mycorrhizal colonization.

On September 9, 2013 we mixed 5 mg of 99 atom-% ^{13}C labeled starch (Cambridge Isotope Laboratories, Tewksbury, MA, USA) into 30 mL of deionized water and injected the mixture evenly into the mesocosm soil profile. We chose the amount of label to track carbon through microbial metabolism, but not fertilize the microbial community (Zak & Kling 2006; Throckmorton *et al.* 2012). At the same time, to control for water addition and disturbance, we injected control mesocosms with 30 mL of deionized water. After four days on September 13, 2013 we measured soil respiration using a LI-6400XT and soil chamber attachment (LiCOR Instruments, Lincoln, NE, USA). To collect gas samples for ^{13}C analysis we capped each mesocosm with a 5 cm PVC cap equipped with a rubber septum. After 20 minutes, we extracted four 15 mL gas samples over a period of 10 minutes from each mesocosms using a syringe and needle. We also collected four site-level samples of ambient air to measure the ambient ^{13}C in the background atmospheric CO_2 . The four $^{13}\text{CO}_2$ samples collected from each core and the average $^{13}\text{CO}_2$ background measurement were used to construct a Keeling plot. The Keeling plot enabled us to calculate the $\delta^{13}\text{C}$ of the source of the carbon substrates metabolized by the soil microbial community (Pataki, Bowling, & Ehleringer 2003). We injected each collected sample into an individual 12 mL Exetainer vacuum vial (Labco Limited, Lampeter, Ceredigion, UK). Samples were analyzed at the UC Davis Stable Isotope Facility (Davis, CA USA) for ^{13}C in CO_2 using a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE USA).

On September 13, 2013 after the $^{13}\text{CO}_2$ samples were collected and respiration was measured, the mesocosms were harvested and kept cool until the soils were processed in the laboratory within 48 hours. When appropriate, data are shown on an oven-dry mass basis. We determined gravimetric water content (GWC) for each mesocosm by oven drying ~15g of field-moist soil at 105°C for 48 hours (Robertson *et al.* 1999).

We assayed soil microbial enzymes that are important in the carbon cycle: β -glucosidase, cellobiohydrolase (hydrolytic), phenol oxidase, and peroxidase (oxidative). Hydrolytic enzymes break cellulose down into glucose, while oxidative enzymes degrade aromatic compounds. Enzymes were measured on field-moist soil within 48 hours of collection using standard methods (see Saiya-Cork, Sinsabaugh, & Zak 2002). Briefly, we homogenized 1 g of field-moist soil with 125 mL of 0.5 M sodium acetate buffer (buffer, pH 5) for 2 minutes with an immersion blender. Soil was mixed with 50 μ L of methylumbelliferyl (MUB)-linked B-glucoside, MUB-linked cellobioside, 3,4-Dihydroxyphenylalanine (L-DOPA), or 10 μ L hydrogen peroxide as substrates for enzymes to act on with eight analytical replicates. For hydrolytic enzymes we filled a black 96 well plate with a blank of buffer only, a reference standard of buffer, and non-linked MUB, a negative control of buffer and MUB-linked substrate, and three replicates each of the soil slurry with non-linked MUB, buffer, or MUB-linked substrate. For oxidative enzymes we filled a clear 96-well plate with buffer only, a reference standard of buffer and L-DOPA substrate, three replicates of soil slurry and buffer, and six replicates of soil slurry and L-DOPA substrate. We incubated the 96-well plates at room temperature for 2 hours (hydrolytic enzymes) or 24 hours (oxidative enzymes) in the dark before analyzing them on a fluorimeter/spectrophotometer (Synergy HT, Biotek Inc, Winooski, VT). We measured fluorescence of hydrolytic enzymes at an excitation of 365 nm and an emission of 450 nm and absorbance of oxidative enzymes was read at 460 nm. Potential enzymatic activity is expressed in units of $\text{nmol h}^{-1} \text{g}^{-1}$ dry soil.

We analyzed microbial biomass-carbon using a chloroform fumigation-extraction described by Vance, Brooks, & Jenkinson (1987). One 15 mL field-moist soil sample was extracted with 50 mL of K_2SO_4 by shaking on a reciprocal shaker (Eberbach Co., Ann Arbor, MI) for 1 h. We filtered the extraction through Whatman # 1 filter paper previously leached with 100 mL DI water. The extraction was stored at -4°C until analysis. A second 15 mL paired soil sample was fumigated with 30 mL of chloroform in an evacuated glass desiccator at room temperature for 5 days and then carbon was extracted as before. We measured microbial biomass-carbon on a total organic carbon analyzer (TOC-V CPH Total Organic Carbon Analyzer, Shimadzu Scientific Instruments,

Columbia, MD) by calculating the fumigated sample minus the unfumigated sample and multiplied by a correction factor of 0.38 (Voroney, Winter, & Beyaert 1993).

We measured mycorrhizal hyphal and root biomass within two weeks of collection. Live fine and coarse roots were removed, dried at 60°C for ~48 h and weighed to determine root biomass. We determined mycorrhizal hyphal biomass by extracting hyphae from the incubated in-growth bags (Wallander *et al.* 2001) using standard floating techniques (Wallander, Goransson, & Rosengren 2004). Hyphae were stored at -20°C and hyphal biomass was freeze-dried to determine weight (Olsson & Wilhelmsson 2000). Hyphal biomass is reported as mg of hyphal biomass per g of sand contained in the in-growth bag (Wallander, Goransson, & Rosengren 2004).

We identified mycorrhizal taxa by sequencing hyphae collected in 5 out of the 30 hyphal in-growth bags. Hyphae were isolated by floating and freeze-drying techniques described above. We extracted DNA from the freeze-dried hyphal tissue (PowerSoil DNA Isolation Kit, MoBio, Carlsbad, CA). DNA was diluted 1:10 with autoclaved DI water prior to being amplified by polymerase chain reaction (PCR) using goTAQ polymerase (Promega, Madison, WI). To target fungal DNA we used ITS-1F and ITS-4 forward and reverse primers. The PCR reaction followed a standard temperature cycling protocol (White *et al.* 1990). We verified PCR products by running them on an electrophoresis gel and we purified them with a PCR clean-up kit (QIAquick PCR Purification kit, Qiagen, Valencia, CA). A single band was visible on the gel, thus we were able to sequence the sample using Sanger sequencing methods (Molecular Biology Resource Facility, University of Tennessee, Knoxville, TN). Sequences were trimmed in Sequencher 5.3 software (Gene Codes Corporation, Ann Arbor, MI) where we aligned the forward and reverse sequences to reduce ambiguous nucleic acid assignments. We then used the BLAST function in Genbank (accessible at ncbi.nlm.nih.gov/genbank/) to match our sequences with taxa identified in the online database. We report only information retrieved using sequences with >98% similarity.

To separate mineral-associated organic matter (MOM) and particulate organic matter (POM), we used a physical fractionation method (Six, Elliot, & Paustian 2000). We weighed 10 g of air-dry and sieved soil in a 10 mL specimen cup with ten 4 mm

glass beads and 40 mL of water and then agitated the slurry on a reciprocal shaker (Eberbach Co., Ann Arbor, MI) for 18 hours. After agitation we poured the soil slurry through a 53 μm screen and rinsed the sample with deionized water to remove all traces of MOM from POM. Solids greater than 53 μm were classified as POM and those smaller than 53 μm were classified as MOM (Six *et al.* 2002). The solutions were oven-dried, ground with mortar and pestle, and analyzed for total carbon content at UC Davis using an Elementar Vario EL Cube or Micro Cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Statistical analyses

First, we tested to see if our experimental designed worked to reduce root and mycorrhizal biomass and if our experimental treatment (mesh size) altered soil properties like soil moisture content. We used a one-way ANOVA to test if our treatments (+R+M, +M-R, -R-M) effectively excluded roots and mycorrhizae and if the different mesh sizes influenced soil moisture using R (R Development Core Team 2011; Zar 1999). Our experimental treatments worked to reduce root and mycorrhizal presence, however the amount that roots and mycorrhizae were reduced varied significantly across our exclusion treatments. Next, we wanted to test if adding ^{13}C starch to the soil primed the soil community. If the response variables for the cores we added starch to were significantly different than the cores we added water to, then we would have to analyze these sets of cores as unique treatments. Using t-tests, we compared microbial activity (enzymes and respiration) and soil carbon pools (microbial biomass, POC, MOC, DOC) in mesocosms where starch was added with mesocosms that only received water (controls). Data were tested for normality using the Shapiro-Wilk test and transformed accordingly and analyzed using R (R Development Core Team 2011). Finally, because root biomass and mycorrhizal biomass varied significantly within our treatments, we used generalized linear models (GLM) to examine the effect of root biomass, mycorrhizal biomass, and the interaction between root and myocrrhizal biomass on soil carbon processes (Zar 1999). Our ^{13}C labeled starch addition did not alter microbial activity, thus we included the +starch and +water control cores in the

analyses for response variables that we did not need to track ^{13}C . GLM models with a poisson distribution were fit separately for our response variables: gravimetric water content, enzyme activity, respiration, microbial activity, microbial biomass, POC, MOC, and DOC. We conducted a separate GLM with data from the cores that received the ^{13}C starch addition to test for the effects of root and mycorrhizal biomass on $^{13}\text{CO}_2$ in mesocosms.

Model simulations

To explore how our experimental treatments might impact soil carbon dynamics over longer time periods (30 y), we ran simulations in the Microbial-Enzyme-mediated Decomposition (MEND) model (Wang, Post, & Mayes 2013) using parameters obtained from our experiment. The MEND model simulates C fluxes from organic matter in soil decomposed by extra-cellular enzymes produced by microbes. It integrates measurable pools of physically defined particular organic carbon (POC), mineral-associated organic carbon (MOC), and dissolved organic carbon (DOC) with microbial carbon biomass (MBC) and microbial exo-enzymes. The model does not explicitly include root and mycorrhizal fungi; however, we implicitly include roots and mycorrhizal fungi by changing microbial physiological parameters that are related to enzyme activity in soil. We used the measured differences in enzyme activities from the experimental treatments to adjust the relevant parameters in different model simulations. Among the parameters, maximal specific decomposition rate (V_{max}), half-saturation constant (K) of Michaelis-Menten kinetics, microbial turnover rate, and carbon use efficiency were the most sensitive parameters that contribute to uncertainty of model predictions (Wang, Post, & Mayes 2013; Allison, Wallenstein, & Bradford 2010; Wieder, Bonan, & Allison 2013). Of these parameters, enzyme activity rates would most clearly affect V_{max} . Using the model, we explored if the difference in V_{max} alone would cause changes in future soil carbon dynamics. We assumed the half-saturation parameter was constant because it is a function of soil texture (Sulman *et al.* 2014; Wieder, Bonan & Allison 2013) and texture varied little at the site (Hanson *et al.* 2001). Soil pH was slightly acidic (pH 4-5) and the bulk density of the B-horizon was $1.4\text{-}1.5\text{ g cm}^{-3}$ (Jardine *et al.* 2006).

We may not be able to observe changes in soil carbon pool sizes in our experimental time frame, thus simulation modeling is necessary to extrapolate to longer time scales (30 y). By calculating the relative differences in enzyme activities among exclusion treatments from the experiment, we were able to simulate the long time period relative differences of soil carbon dynamics that emerged as changes in POC and MOC pools. Although we recognize the importance of a mycorrhizal fungal feedback to plant productivity via increased nitrogen uptake, this was not the primary focus of this study and we were unable to include it in the model. We assumed that roots and mycorrhizal fungi affected microbial activity without changing soil properties such as texture (but see Six *et al.* 2006).

We parameterized the MEND model using measured POC, MOC, MBC, and DOC pool size data as initial steady state variables. We used the SCEUA (Shuffled Complex Evolution at University of Arizona) algorithm (Duan, Sorooshian, & Gupta, 1992; Wang, Xia, Chen 2009) to estimate V_{max} , K , microbial turnover rate, and microbial carbon use efficiency. All unmeasured parameters in the MEND model were derived from the literature (Wang *et al.* 2012). The experimental treatment group that allowed root and mycorrhizal fungi access to soil was the null group in our exercise because roots and mycorrhizal fungi are commonly found together in the soil matrix. The parameters in the null group were forced to fit measured pool size data. Other treatment groups shared parameters with the null group, except the parameter V_{max} , maximum specific decomposition rate. We assumed cellobiohydrolase and peroxidase activities represented the maximum specific decomposition rate of POC and MOC, respectively, in our treatments. We chose these enzymes to parameterize POC and MOC decomposition because in our model POC and MOC vary in residence time and cellobiohydrolase decomposes cellulose, which has a relatively fast residence time, while peroxidase decomposes lignin, which has a relatively slow residence time. We maintained constant inputs of carbon (i.e., similar levels of soil litter inputs) to the system when simulating the different exclusion treatments. We conducted a Monte Carlo resampling from cellobiohydrolase and peroxidase data to calculate the ratios of maximum specific decomposition rates among the treatments. We calculated the

analytic solution to the MEND model at steady-state, which was reached at approximately 30 years of simulation. Simulations were carried out using Matlab 2012a (The MathWorks, Inc., Natick, Massachusetts, USA). To determine the role of roots and mycorrhizal fungi on soil carbon storage, we compared the response ratio of POC and MOC in simulations lacking either root- or mycorrhizal fungal-mediated enzyme activity to simulations with root- and mycorrhizal fungal-mediated enzyme activity. The response ratio was calculated as the POC (or MOC) value at steady state in a model without roots (i.e., -roots +myc) or mycorrhizal fungi (i.e., -roots -myc) divided by the POC (or MOC) value at steady state in a model with roots and mycorrhizal fungi (+roots +myc). We statistically compared the response ratios using a Mann-Whitney U test for POC and MOC responses, separately. A positive response ratio indicated greater carbon storage in soil pools. For example, a response ratio of 2 would indicate that a model that lacked either roots or mycorrhizal fungi would result in 2× more carbon storage compared to a model that contained both roots and mycorrhizal fungi. A significant p-value ($\alpha = 0.05$) indicates a difference in carbon storage in models with and without mycorrhizal fungi.

Results

Experimental results

While we were able to reduce root and mycorrhizal biomass in our mesocosm experiments, the treatments varied in their effectiveness. We did not find significant differences in root ($n = 10$, $F_{2, 27} = 3.06$, $P = 0.06$) or mycorrhizal biomass ($n = 10$, $F_{2, 27} = 0.53$, $P = 0.60$) among mesh exclusion treatments. Overall, the root exclusion mesocosms contained approximately 50% less fine root biomass (5 μm mesh: 71.15 mg $\pm$ 63.99; 55 μm mesh: 75.96 mg $\pm$ 82.97) than root access mesocosms (1.45 mm mesh: 134.37 mg $\pm$ 120.58; $P = 0.05$, $F = 3.31$). Mycorrhizal exclusion mesocosms reduced mycorrhizal biomass by approximately 50% (5 μm mesh: 2.38 mg $\pm$ 5.78) compared to mesocosms containing mycorrhizae but excluding roots (55 μm mesh: 4.93 mg $\pm$ 10.24) and by 60% compared to mesocosms containing mycorrhizae and roots (1.45 mm mesh: 6.46 mg $\pm$ 11.92). Gravimetric water content was not altered by mesh size ($n = 8$,

$F_{2,22} = 0.01$, $P = 0.99$). Given the effect of our treatments varied and our response variables were not normally distributed, we used GLM models to explore how root biomass, mycorrhizal biomass, and their interaction altered soil carbon processes.

We successfully traced ^{13}C into CO_2 four days after injecting the labeled starch into mesocosm soil that received the labeled starch addition compared to mesocosms receiving water ($n = 15$, $t = -3.41$, $P = 0.002$). Total soil respiration did not differ between ^{13}C -starch-added and water-control mesocosms ($n = 5$, $t = 1.16$, $P = 0.27$). The addition of starch to the mesocosms did not alter POC, MOC, or DOC ($n = 4$, $t = 0.19$, $P = 0.85$; $n = 5$, $t < 0.01$, $P = 0.99$; $n = 11$, $t = -1.91$, $P = 0.07$ respectively). Similarly, MBC did not vary with ^{13}C starch addition compared to water addition controls ($n = 15$, $t = -1.40$, $P = 0.18$). The addition of ^{13}C -starch also did not affect the activity of cellobiohydrolase ($n = 11$, $t = 0.40$, $P = 0.70$), β -glucosidase ($n = 13$, $t = -0.10$, $P = 0.92$), phenol oxidase ($n = 13$, $t = 0.63$, $P = 0.54$), or peroxidase ($n = 13$, $t = 0.07$, $P = 0.95$) relative to cores where only water was added, thus we were able to combine the starch addition and control mesocosms in our microbial activity and soil carbon pool analyses.

In general, root biomass and mycorrhizal biomass interacted with one another to alter soil enzyme activity (Fig. 2). β -glucosidase activity was highest when mycorrhizal and root biomass was high and mycorrhizal biomass was present; however rates were low when both mycorrhizae and roots were absent ($n = 23$, $\chi^2 = 203.31$, $P < 0.001$; Fig 2a, Table 1). Overall, cellobiohydrolase activity was highest when root and mycorrhizal biomass were highest; however when mycorrhizal biomass was low the impact of root biomass on cellobiohydrolase activity varied ($n = 22$, $\chi^2 = 43.30$, $P < 0.001$, Fig 2b). Peroxidase activity was high when mycorrhizal fungal biomass was present; however, it was low when mycorrhizal biomass was absent and this was independent of root biomass ($n = 28$, $\chi^2 = 233.99$, $P < 0.001$; Fig 2c). There was not a significant interaction between root and mycorrhizal biomass on phenol oxidase activity ($n = 26$, $\chi^2 = 1.86$, $P = 0.17$; Fig 2d); however, phenol oxidase activity was high when root biomass was high ($n = 26$, $\chi^2 = 302.29$, $P < 0.001$, Table 1).

Root and mycorrhizal biomass had variable impacts on soil organic carbon pools and fluxes in our short experimental time-scale. There was a significant interaction

between root and mycorrhizal biomass where ^{13}C in CO_2 respiration was highest where root biomass was high and mycorrhizal biomass was low ($\chi^2 = 196.44$, $P < 0.001$; Fig 3a). The POC pool was positively correlated with the interaction between root and mycorrhizal fungal biomass ($n = 9$, $\chi^2 = 5.15$, $P = 0.02$; Table 1; Fig 3b). POC was high where mycorrhizal biomass was at a mid-level and it was lowest where root biomass was high. The MOC pool was not correlated with the interaction between root and mycorrhizal biomass ($n = 13$, $\chi^2 = 0.05$, $P = 0.83$; Fig 3c), but it was negatively correlated with root biomass ($n = 13$, $\chi^2 = 3.98$, $P = 0.05$; Table 1). DOC ($n = 26$, $\chi^2 = 0.82$, $P = 0.37$; Table 1), microbial biomass carbon (MBC) ($n = 30$, $\chi^2 = 0.02$, $P = 0.89$; Fig 3d), and GWC ($n = 25$, $\chi^2 < 0.001$, $P = 0.98$) did not vary with root biomass, mycorrhizal biomass, or their interaction.

We isolated two ectomycorrhizal taxa from hyphae collected in a subset of hyphal in-growth bags ($n = 5$). We identified *Tomentella* spp. in 80% of the samples analyzed and an unknown species of the family Sebacinaceae in 20% of the samples analyzed. *Tomentella* was found in all three exclusion mesocosm types and Sebacinaceae was found in a single mesocosm with the smallest mesh designed to exclude roots and mycorrhizal fungi. We did not identify any arbuscular mycorrhizal species in any of our samples.

Model simulation results

With our short-term *in situ* mesocosm experiments we found that microbial enzyme activity varied with root and mycorrhizal fungal presence. However, we were interested if root and mycorrhizal abundance would influence soil carbon dynamics over a longer-term (30 y) period. Therefore, we used the MEND model to simulate carbon fluxes in soils by adjusting V_{max} to account for the observed enzyme activities from our experiment. Thus, we were able to compare carbon dynamics in soil systems containing roots and mycorrhizal fungi to soil systems without roots and mycorrhizal fungi. Given the enzymes measured in our experiment varied in their response to root and mycorrhizal abundance, this modeling approach likely represents the maximum

potential for differences among our treatments and may not represent all of the possible outcomes observed in the experimental treatments.

To parameterize V_{max} , we calculated mean enzyme rates for ten mesocosms representing each biomass grouping: high root and high mycorrhizal biomass, low root and high mycorrhizal biomass, and low root and low mycorrhizal biomass. Based on the measured mean cellobiohydrolase value ratio among the biomass groups, we reduced V_{max} by 60% for the model with mycorrhizae but no roots and by 26% for the model without roots or mycorrhizae to calculate POC. Based on peroxidase values, we reduced V_{max} by 31% for the model with mycorrhizae but no roots and by 36% for the model without roots or mycorrhizae. When enzyme activities and V_{max} were adjusted to levels observed when root and fungal biomass were low in simulations, microbial processing of soil carbon was reduced, leading to increased MOC and POC pools after 30 years. We report the response ratio of simulated MOC and POC in a model where root and mycorrhizal presence modifies enzyme activity rates compared to a model where either root or root and mycorrhizal fungal effects were reduced (Fig. 4). MOC increased approximately 2× in soil when roots biomass was low compared to the control model with high root and mycorrhizal biomass, but soil MOC was not sensitive to relative amount of root and mycorrhizal biomass ($P = 0.59$, $z = -0.53$). POC increased in soil approximately 4×, with a range of 1× to 10×, when enzyme activities and V_{max} were adjusted to levels observed when mycorrhizal biomass was high and root biomass was low, compared to adjustments assuming both root and mycorrhizal biomass were high ($P < 0.001$, $z = -17.21$). Overall, this simulation result suggested that low biomass of either roots or mycorrhizal fungi in soil modifies enzyme activity rates such that pools of carbon can build up over time (>30 y).

Discussion

Roots, mycorrhizal fungi, and their associated microbial communities play an important role in regulating the flux of carbon from the soil to the atmosphere (Gadgil & Gadgil 1975; Bardgett & Wardle 2010). We predicted that root biomass and mycorrhizal biomass would interact in an additive way to increase microbial metabolism of soil

carbon; however, this is not what we found. The metabolism of ^{13}C labeled starch was highest when root biomass was high and mycorrhizal biomass was low, suggesting that interactions among roots, mycorrhizae, and the soil community may not always be positive. Translating our experimental results into the MEND model suggested that roots and mycorrhizal fungi could differentially affect pools of soil carbon over 30 y time frames: roots enhanced decomposition processes leading to a decline in storage of carbon in POC and MOC pools, and mycorrhizal fungi buffered the effect of roots on POC by reducing the overall effect on decomposition processes leading to greater storage of carbon in POC.

Roots and mycorrhizal fungi add carbon to the soil (Bais *et al.* 2006; Kuzyakov 2010; Phillips *et al.* 2012), thus we predicted that across all the carbon-cycling enzymes we measured, enzyme activity would be highest when root and mycorrhizal fungal biomass were high, intermediate when mycorrhizal fungal biomass and/or root biomass were low, and lowest when roots and mycorrhizal fungi were both excluded. However, root and mycorrhizal fungal presence did not additively increase microbial activity or the microbial decomposition of soil carbon. Instead, β -glucosidase activity, which is involved in cellulose decomposition (Baldrian & Valášková 2008), was high where root biomass was high and mycorrhizal biomass was low. Cellulose, a carbon rich and nutrient poor compound, may not be degraded as quickly when mycorrhizal fungi are present. Mycorrhizal fungi receive carbon from their associated plant roots in exchange for nutrients, thus they do not need carbon from the soil to build their own biomass (Smith & Read 2008). Previous work shows that roots and mycorrhizal fungi can manipulate microbial activity based on limiting resources (Bais *et al.* 2006). In our temperate forest ecosystem, nutrients found in lignin but not cellulose (e.g., nitrogen) may limit plant growth because nitrogen typically limits growth in temperate ecosystems (Xu, Thornton, and Post 2013). Phenol oxidase, a lignin-degrading enzyme, was not affected by the interaction between roots and mycorrhizal fungi, suggesting their role is not generalizable across all enzymes, thus limiting their role in regulating decomposition. Other factors such as soil pH or texture may also be important drivers of enzyme activity, but these likely did not vary among our treatments. Therefore, the effect of

mycorrhizal fungi on microbial activity may depend on the substrate being decomposed, by the soil community present, and by the abiotic conditions of the ecosystem.

Taken together, our results suggest that roots and mycorrhizal fungi affect labile pools of carbon such as POC more than recalcitrant pools of carbon such as MOC, a hypothesis supported by our model simulations. We speculated that mycorrhizae may promote lignin degradation in order to access nitrogen and slow cellulose degradation to maintain the carbon limitation of the free-living microbes they compete with, although the exact mechanisms of these interactions remain to be tested (Floudas *et al.* 2012, Lindahl & Tunlid 2014). We found that cellobiohydrolase, a cellulose-degrading enzyme, was negatively correlated with mycorrhizal biomass and that peroxidase, a lignin-degrading enzyme, was positively correlated with mycorrhizal biomass, which supports this idea. These correlations suggest three things: (1) that mycorrhizal fungi promoted the microbial decomposition of compounds in order to increase their nitrogen acquisition (Lindahl *et al.* 1999), (2) mycorrhizal fungi were active in lignin decomposition (Talbot, Allison, & Treseder 2008; Wolfe, Tulloss, & Pringle 2012), or (3) mycorrhizal fungi were suppressing microbial growth (de Boer *et al.* 2005). These three possible mechanisms are not mutually exclusive; for example, mycorrhizae may suppress microbial biomass (e.g., using antibiotics, see de Boer *et al.* 2005) in order to gain greater access to the nitrogen bound in lignin. Overall, mycorrhizal fungal biomass in this study affected soil carbon dynamics, suggesting their impact may not mirror that of roots, and they may be reducing the activity of some of the free-living microbes in the community.

The interaction between mycorrhizal fungi and members of the free-living microbial community may be species-specific (de Boer *et al.* 2005). We found that the interaction between roots and mycorrhizal fungi had a negative effect on β -glucosidase activity while the interaction had a positive effect on cellobiohydrolase activity — both hydrolytic enzymes — suggesting that mycorrhizal fungi vary in the way they interact with roots. Mycorrhizal species vary in their architecture and ability to grow away from roots to explore the soil matrix (Agerer 2001). We predicted that the proximity of mycorrhizal hyphae to roots, a mycorrhizal species-specific trait, might be important in regulating mycorrhizae-microbe interactions (Churchland & Grayston 2014). However,

our data suggested that communities of mycorrhizae were similar across our treatments, consisting primarily of ectomycorrhizal *Tomentella* spp. and Sebacinaceae (Selosse, Bauer, & Moyersoen 2002). To tease this pattern apart, further experimental work exploring how mycorrhizal communities located near roots and away from roots differentially affect activity of free-living microbes is needed. An alternative explanation for the positive correlation between cellobiohydrolase activity and the root-mycorrhizal interaction is that members of the microbial community are obtaining carbon from the more labile root exudates rather than from the cellulose pool in the soil. This second hypothesis was supported by the total soil respiration data, which did not vary with root or mycorrhizal biomass. Because total respiration was similar when roots were present and absent, members of the free-living microbial community may have used a different carbon source without changing the total amount of carbon metabolized.

Roots can provide simple carbon compounds to the free-living microbial community (Kuzyakov 2010), which may explain the lower utilization of ^{13}C starch when roots and mycorrhizal fungi were present. Therefore, we predicted that the free-living microbial community would metabolize the added starch, or soil carbon, at a higher rate when mycorrhizal fungi and roots were both present to 'prime' the free-living community with easily degradable carbon. We did not, however, find enhanced respiration of ^{13}C starch or total soil respiration when roots were present. This is in contrast to findings of girdling studies where soil respiration was reduced by 50% when inputs to the soil from tree roots ceased (Hogberg *et al.* 2001; Hogberg & Read 2006; Subke *et al.* 2011). Supplementary sugars from plant roots do not universally aid microbes in soil carbon decomposition and this relationship varies with exudate concentrations (de Graaff *et al.* 2010). Thus, microbial activity could be stoichiometrically constrained such that root exudates are not supplying the elements microbes require to break down soil organic carbon, thus priming may not universally increase the microbial breakdown of soil carbon (Drake *et al.* 2013).

Using the MEND model (Wang, Post, & Mayes 2013), we adjusted the maximum decomposition rate (V_{max}) based on our experimental enzyme activity results to qualitatively compare the effects of roots and mycorrhizal fungi on MOC and POC pool

dynamics. Our simulations suggested that excluding roots slowed decomposition and enabled the build-up of carbon in both pools while excluding mycorrhizal fungi contributed to a greater build-up of POC relative to MOC. The simplest explanation for an increase in MOC and POC concentrations is that less carbon was being metabolized and respired by the free-living microbial community in treatments where roots were absent. This hypothesis was supported by the lower enzyme activity rates measured in our experiment and the accompanying V_{max} . Mycorrhizal fungi have different impacts on MOC and POC pools and our simulations suggest that mycorrhizal fungi reduce the pool of POC pool while having a negligible effect on MOC.

The variability in simulations in the model with mycorrhizae but without roots was high; indicating that variation in the maximum decomposition rate (V_{max}) estimated from soils without roots does not correlate with soils that include roots. In other words, mycorrhizal fungi away from roots could select for microbial communities that are different from microbial communities that interact with both roots and fungi. This hypothesis could be tested experimentally by comparing the microbial community before and after the removal of roots from the soil matrix; however, this design would likely disturb the intact soil communities. The variability in microbial communities is high, thus conducting an unpaired test (soils with and without roots) in an experiment would require a large sample size to overcome the large background variability in microbial community composition. While our model results are interesting, they were based on the measured activity of two enzymes: peroxidase and cellobiohydrolase activity. Therefore, we recommend parameterizing future models using a suite of hydrolytic and oxidative carbon-degrading enzymes. Additionally, including other factors such as changes in microbial carbon use efficiency, microbial turnover rates (Blagodatskaya *et al.* 2014), variance in soil texture (Six *et al.* 2006), and changes in carbon inputs (Langley, Chapman, & Hungate 2006, de Boer *et al.* 2005) would enhance model inference. Turnover of roots and mycorrhizal fungi could contribute to carbon input to soil. We assumed constant carbon input among treatments, which may overestimate soil carbon pool sizes in simulations without roots. Our model may also underestimate the degree to which mycorrhizal fungi contribute to soil carbon storage for two reasons:

(1) turnover of recalcitrant mycorrhizal tissues could result in a slower decomposition rate (Langley, Chapman, & Hungate 2006), and (2) mycorrhizae are known to alter soil texture on a fine scale by increasing soil aggregate formation (Six *et al.* 2006). Currently, the MEND model does not have separate pools or activity rates explicitly representing different soil community members (e.g., roots, mycorrhizal fungi, free-living microbes), but our findings suggest that future model development could be warranted. Overall, our model exercise provides a general approach to scale enzymatic data to soil carbon pools over time.

The ecosystem-level outcomes of root-mycorrhizae-microbe interactions are complex and might differ with tree and mycorrhizal biodiversity, community structure, or under different climatic and environmental limitations (Chapin *et al.* 2009). This study was conducted in a temperate forest over a single growing season. In our ecosystem, ectomycorrhizal fungi are common relative to other ecosystems across the globe (Tedersoo *et al.* 2014); thus, the results of our study may vary by location or time (Cregger *et al.* 2014; Talbot *et al.* 2014). For example, mycorrhizal species can differ in their rates of resource acquisition (Smith & Read 2008), which could affect microbial abundance through interference competition. Similarly, through their diverse hyphal architectures (Agerer 2001) mycorrhizal species may differentially facilitate the free-living microbes by providing habitat and refuge from stressors like drought, an interaction that may vary over time (de Boer *et al.* 2005; Frey-Klett *et al.* 2011). Identifying how shifts in the mycorrhizal community are related to changes in microbial community and ecosystem function over time and across different ecosystems is a potential next step in understanding how root-mycorrhizal-microbial interactions influence the carbon cycle (Classen *et al.* 2015). Our study demonstrates that roots and mycorrhizal fungi differentially affect carbon dynamics. Complex interactions among roots, mycorrhizal fungi, and free-living microbes may explain some of the observed heterogeneity in soil decomposition and mineralization rates (Hinsinger *et al.* 2005).

Data Accessibility

Data collected in this study are archived at Dryad (<http://dx.doi.org/10.5061/dryad.pb271>).

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Appendix: Figures

Figure 2.1. Conceptual diagram illustrating hypothetical outcomes of root-mycorrhizal-microbial interactions. Our experimental mesocosms target three distinct and naturally occurring types of soil communities: communities with roots (dark colored) and mycorrhizal fungi (light colored) (+roots +myc), without roots (-roots +myc), and without either (-roots -myc). We examined the response of soil respiration (arrows, size indicates magnitude of flux), enzyme activity (boxes, + indicates greater magnitude of activity), and storage of soil organic carbon (SOC; darker shading of soil indicates higher magnitude of pool size) to root-mycorrhizal-microbial interactions. We predicted that decomposition processes would be greatest where roots and mycorrhizal fungi are present due to exudates stimulating microbial activity and that those processes would be reduced when roots or mycorrhizal fungi were absent from the soil community. We also predicted that SOC pools would be inversely related to decomposition processes: where decomposition was expected to be high, we would expect SOC pools to be low.

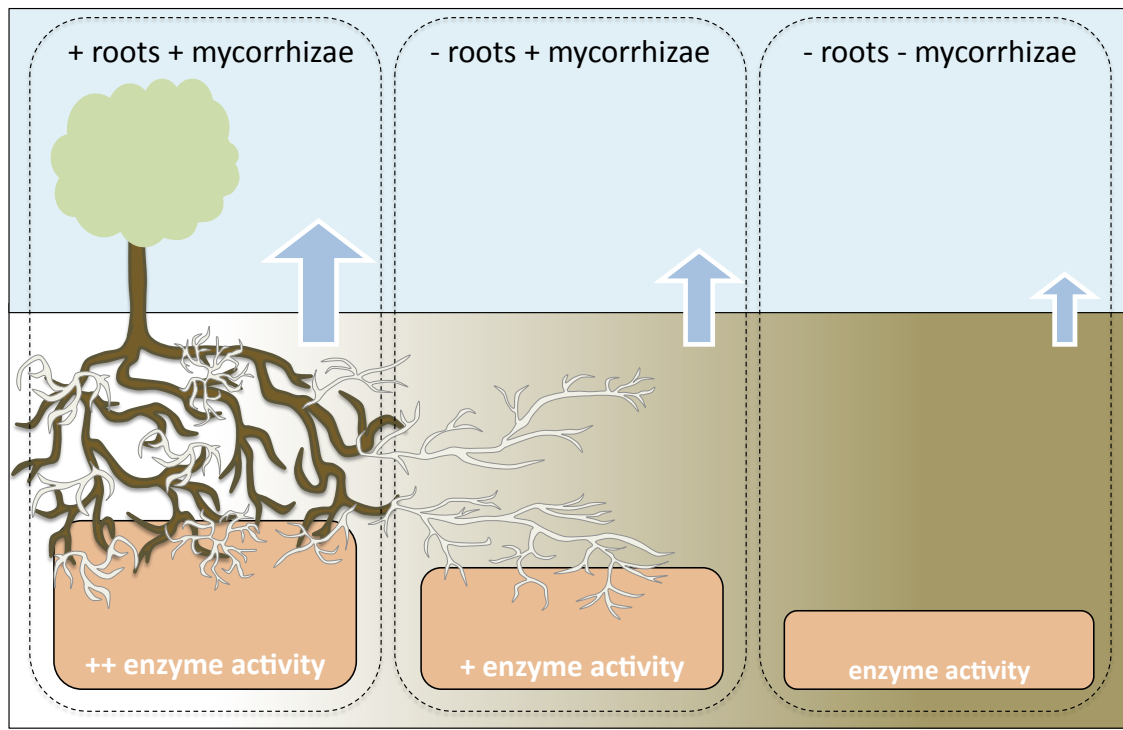


Figure 2.1. Continued.

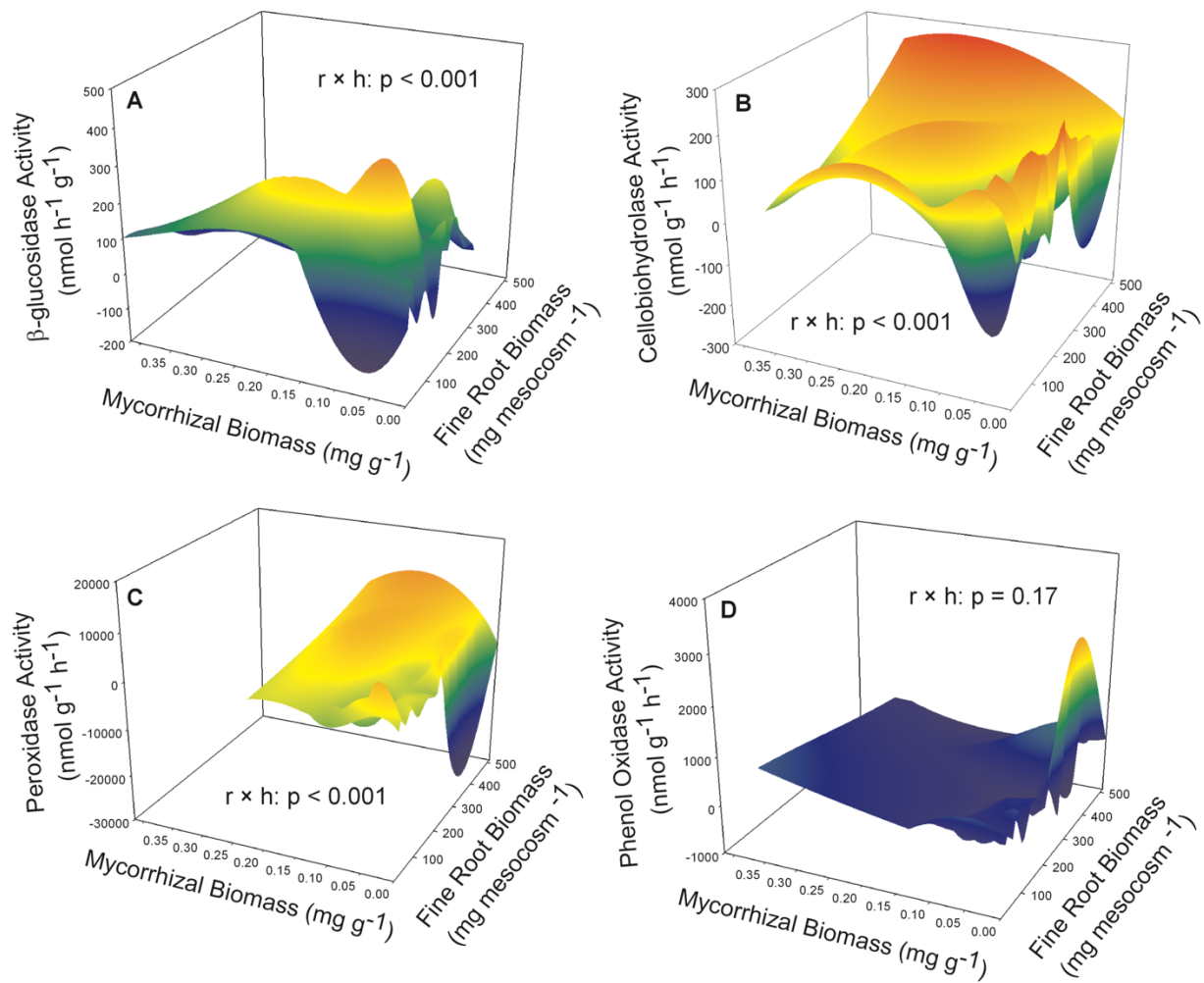


Figure 2.2. Effects of the interaction between root and mycorrhizal hyphal biomass on soil carbon processes and pools. (A) Potential β glucosidase activity ($\text{nmol g}^{-1} \text{h}^{-1}$) was negatively correlated with the interaction of roots and mycorrhizal biomass ($\chi^2 = 203.31$, $P < 0.001$). (B) Potential cellobiohydrolase activity ($\text{nmol g}^{-1} \text{h}^{-1}$) was positively correlated with the interaction between roots and mycorrhizal biomass ($\chi^2 = 43.30$, $P < 0.001$). (C) Potential peroxidase activity ($\text{nmol g}^{-1} \text{h}^{-1}$) was positively correlated with the interaction between roots and mycorrhizal biomass ($\chi^2 = 233.99$, $P < 0.001$). (D) Potential phenol oxidase activity ($\text{nmol g}^{-1} \text{h}^{-1}$) had no correlation with the interaction between roots and mycorrhizal biomass ($\chi^2 = 233.99$, $P < 0.001$). Abbreviations: r, fine root biomass; h, mycorrhizal hyphal biomass.

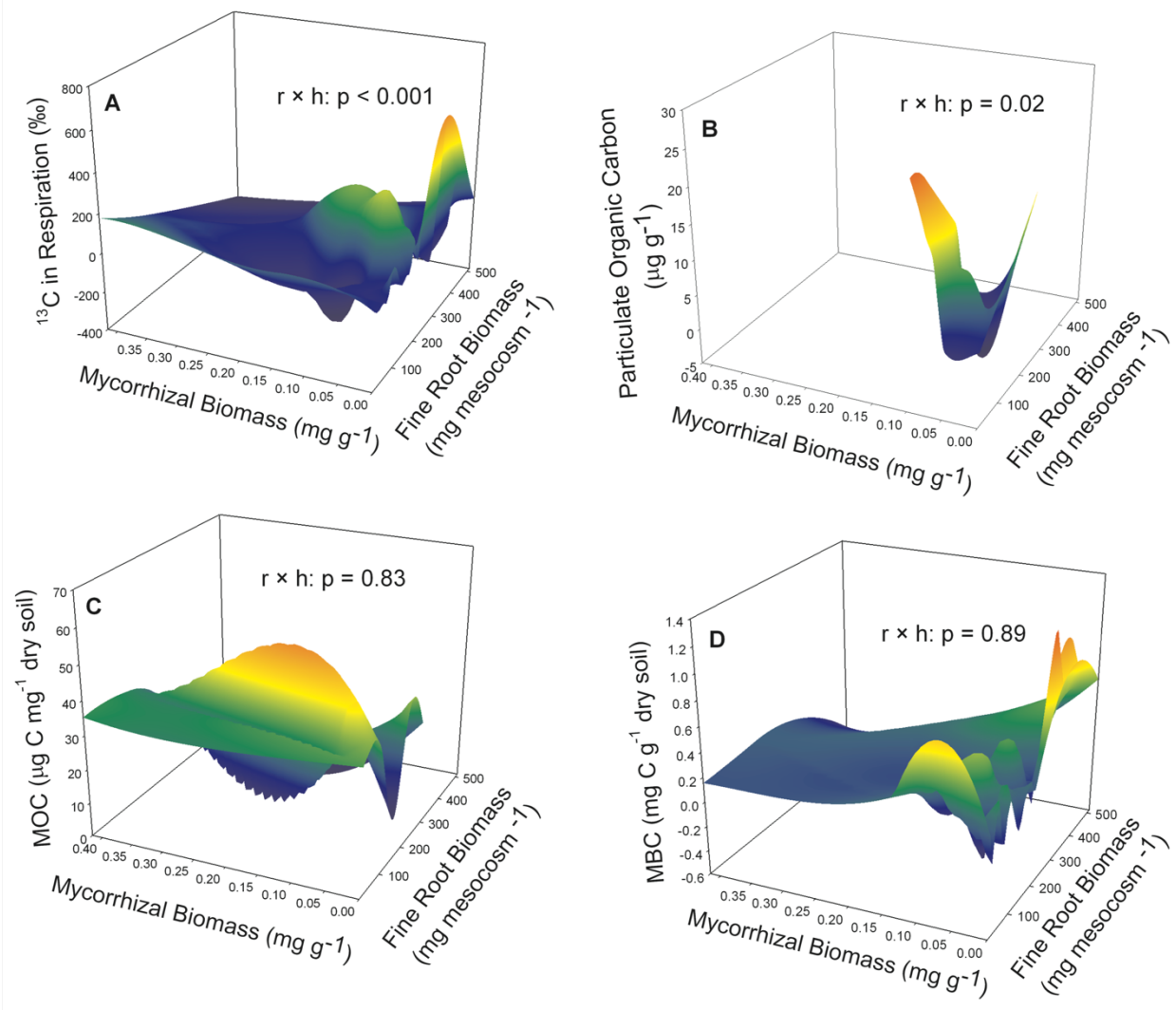


Figure 2.3. Fluxes and pools of soil C are affected by the interaction between root and mycorrhizal biomass. A) Respiration of ^{13}C starch was positively correlated with root and mycorrhizal biomass ($\chi^2 = 196.44$, $P < 0.001$). B) Particulate soil organic carbon (POC) was negatively correlated with root and mycorrhizal biomass ($\chi^2 = 5.15$, $P = 0.02$). There was no correlation between root and mycorrhizal biomass and mineral organic carbon (C; MOC; $\chi^2 = 0.05$, $P = 0.83$) or microbial biomass carbon (D; MBC; $\chi^2 = 0.02$, $P = 0.89$). Abbreviations: r, fine root biomass; h, mycorrhizal hyphal biomass.

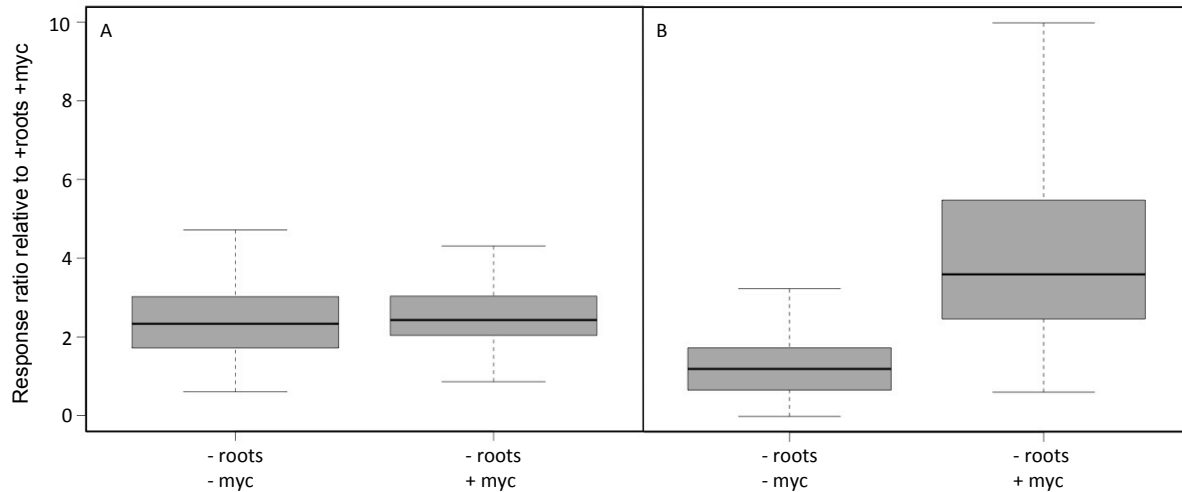


Figure 2.4. Effects of roots and mycorrhizal exclusion on simulated carbon pools. We show mineral (A) and particulate (B) organic carbon (MOC and POC, respectively) at steady state. Boxplots show data from permuted simulations. The black line indicates the median value, the box indicates the upper and lower quartiles and the whiskers indicate the range of data. The response ratio was calculated as the ratio of MOC or POC values obtained in a model with low root biomass and either high or low mycorrhizal fungal biomass (-roots +myc, -roots -myc, respectively) relative to a model containing high root and high mycorrhizal fungal biomass (+roots +myc).

Appendix: Tables

Table 2.1. Generalized linear model results of root and mycorrhizal effects on soil carbon pools and processes. Fine root (r) and mycorrhizal hyphal (h) biomass effects on activity of enzymes, microbial biomass carbon (MBC), particulate OC (POC), mineral OC (MOC), and dissolved OC (DOC) are shown.

Response	Model	Chi Square	P
β-glucosidase	r	39.95	<0.0001
	h	2.06	0.1508
	r × h	203.31	<0.0001
Cellobiohydrolase	r	6.17	0.01
	h	65.10	<0.0001
	r × h	43.30	<0.0001
Peroxidase	r	2168.10	<0.0001
	h	1425.24	<0.0001
	r × h	233.99	<0.0001
Phenol oxidase	r	302.29	<0.0001
	h	0.45	0.50
	r × h	1.86	0.17
MBC	r	0.10	0.75
	h	0.0086	0.92
	r × h	0.016	0.89
POC	r	0.06	0.81
	h	1.66	0.20
	r × h	5.15	0.02
MOC	r	3.98	0.05
	h	0.18	0.67
	r × h	0.05	0.83
DOC	r	0.31	0.58
	h	1.05	0.30
	r × h	0.82	0.37

Chapter 3: Roots stimulate soil carbon decomposition while mycorrhizal hyphae stimulate microbial nutrient acquisition

Abstract

Bacteria and fungi in soil are the primary decomposers of soil organic matter, but biotic factors regulating their activity is poorly understood. Roots and mycorrhizal fungi are known to alter activity of free-living soil microbes. On one hand, roots and mycorrhizal fungi can release exudates that stimulate microbial activity. On the other hand, competition between roots, mycorrhizae, and microbes for nitrogen may suppress microbial activity. Our understanding of the combined effects of roots and mycorrhizal fungi on decomposition activity of free-living microbes is limited. We hypothesized that roots would stimulate microbial activity but mycorrhizal fungi would dampen this effect via competitive exclusion.

We tested our hypotheses in an upland boreal forest by manipulating root and mycorrhizal biomass *in situ* and measuring microbial biomass and several indicators of microbial activity: carbon and nutrient-degrading enzyme activities, soil respiration, and metabolism of a ^{13}C -labeled substrate. Using a structural equation model, we analyzed indirect and direct effects of root and mycorrhizal biomass on microbial biomass and activity. We found that roots directly affect carbon-degrading enzyme activity while mycorrhizal fungi directly affect nitrogen-degrading enzyme activity. Metabolism of ^{13}C -labeled starch was highest where root biomass was low, indicating microbes rely on root-derived C to degrade cellulose. Soil respiration was more strongly affected by a direct effect of microbial biomass than the indirect effect of enzyme activities mediated by microbial biomass. Thus, measurements of enzyme activity may be poor indicators of total release of C from soil. Overall, our study demonstrates that decomposition of organic matter is more strongly influenced by roots and mycorrhizal effects on enzyme activities rather than changes in microbial biomass.

Introduction

Soil microbes are essential to ecosystem processes such as primary production, nutrient cycling, and decomposition (Bardgett and Wardle 2010). Soil organic matter (SOM) decomposition is a critical step in terrestrial carbon (C) fluxes that is executed primarily by soil microbes (Van Der Heijden et al. 2008). Extracellular enzymes excreted by soil microbes decompose SOM by targeting specific organic compounds (Sinsabaugh et al. 2008). Enzymatic activity is sensitive to temperature, pH, and soil moisture (Wagai et al. 2013), and implementation of these mechanisms has improved recent ecosystem C models (Treseder et al. 2012, Todd-Brown et al. 2012, Schimel 2013). However, microbial enzyme activity is also sensitive to interactions among microbes (Moorhead and Sinsabaugh 2006) and among roots, root-associated fungi, and soil microbes (Drake et al. 2013, Clemmensen et al. 2013, Lindahl et al. 2010). Current ecosystem models have not implemented explicit ecological controls on microbial enzyme activity because effects of roots and root-associated mycorrhizal fungi on microbial enzyme activity specific to C and nutrient-rich substrates has yet to be resolved.

Soil microbial activity can be stimulated or suppressed by roots. Plants allocate up to 20% of total net primary productivity (NPP) belowground (Hobbie 2006). Some of the photosynthate allocated belowground is released from roots as sugary exudates, which alleviate microbes from C limitation and increases their activity (Kuzyakov et al 2010); in fact 11.5% – 21.5% of soil respiration is due to root stimulation of microbial activity (Crow et al. 2009). When fresh photosynthetically fixed C from trees is cut off to the microbial community, microbial activity declines (Hogberg et al. 2001, Subke et al. 2011). Soil enzyme activity declined 40% when trees were slowly killed and C flow was reduced to the soil community in a deciduous forest (Brzostek et al. 2015). Interestingly, root exudation may not always increase soil nutrient availability. Over time root exudation may select for a root-exudate dependent soil community (Nazir et al. 2010, Hartmann et al. 2009) that has lower rates of SOM decomposition (Kaiser et al. 2010). In addition, adding C rich substrates to the soil can increase nutrient limitation of the soil microbial community and impede microbial enzyme production and SOM decomposition

(Drake et al. 2013). While mycorrhizal fungi are integral in root-soil interactions, it remains unclear whether mycorrhizal fungi, which scavenge for soil nutrients, increase the N-limitation effects of root exudates.

Mycorrhizal fungal hyphae promote microbial activity in some environments while dampening activity in other environments. For example, chitinase, phosphatase, and phenol oxidase enzymes were twice as active in mycorrhizal hyphal mats compared to non-mycorrhizal hyphal mats in a pine forest (Kluber et al. 2010). In another study across several temperate coniferous and deciduous forests, nutrient-acquiring enzyme activity increased by 10% - 30% compared to surrounding bulk soil (Phillips and Fahey 2006). The increased enzyme activity can be explained by hyphal exudates, which were 2.7 times higher in mycorrhizal compared to non-mycorrhizal hyphal mats (Kluber et al. 2010). Hyphal exudates increase microbial growth and biomass (Toljander et al. 2007) and microbial community diversity (Tedersoo et al. 2009). However, mycorrhizal fungal hyphae can also suppress microbial growth and activity. In a greenhouse experiment, presence of mycorrhizal hyphae reduced bacterial biomass by 30 – 50% (Nuccio et al. 2013). Hyphae compete for resources such as N in soil, and through competitive exclusion they can suppress microbial growth (Nuccio et al. 2013, Veresoglou et al. 2011). Mycorrhizal hyphae can further suppress microbial activity by exuding inhibitory chemicals, slowing microbial activity (measured as thymidine incorporation) by 20 - 50% (Olsson et al. 1996). Mycorrhizal rhizospheres have different bacterial communities from bulk soil (Nuccio et al. 2013), with lower abundances of microbes known to decrease mycorrhizal fungal spore germination and negatively affect hyphal growth (Pivato et al. 2009). The combined effects of mycorrhizal roots and hyphae on microbial decomposition activity remains unresolved.

To understand how root-hyphal interactions influence soil microbial activity and overall ecosystem processes we manipulated root and hyphal access to the soil microbial community, thus generating a gradient of root-hyphal influence on soil processes. We measured enzymatic activity, soil respiration, and metabolism of ¹³C-labeled starch as indicators of microbial activity. We hypothesized that (i) where root biomass was high microbial activity would be high due to stimulating effects of root

exudates, (ii) where hyphal biomass was high microbial activity would be suppressed due to competitive exclusion, and (iii) effects of mycorrhizal roots and hyphae on microbial enzyme activity would be indirect and mediated by their influence on microbial growth and biomass. Understanding the biological regulators of decomposition, the directions of root and hyphal influences, and how they might change with environmental factors will elucidate how changes in biological interactions can alter soil C accrual.

Methods

The field site was located in the Marcell Experimental Forest (47°30'26.73"N, 93°27'15.68") located 40km north of Grand Rapids, Minnesota, USA. The average annual temperature is 3°C, and average precipitation is 78.5cm yr⁻¹. The study site was located in a 40 m × 40 m area within a forest primarily composed of bigtooth aspen (*Populus grandidentata*), trembling aspen, (*Populus tremuloides*) and paper birch (*Betula papyrifera*). The dominant understory plants were bracken fern (*Pteridium aquilinum*), dwarf raspberry (*Rubus pubescens*), round-leaved dogwood (*Cornus rugosa*), and beaked hazel (*Corylus cornuta*). Bigtooth aspen and trembling aspen associate with both ectomycorrhizal (ECM) fungi and arbuscular mycorrhizal (AM) fungi, while paper birch only associates with ECM fungi. *Dogwood spp.* are known to associate with AM fungi. Soils were pH 5.0 ± 0.44 with a bulk density of 1.35 ± 0.45 g cm⁻³ and are fine, sandy loams classified as Warba series (Kolka et al. 2001).

To manipulate root and mycorrhizal biomass we constructed cores made from a 15 cm long, 5 cm diameter PVC pipe. Two 10.5 cm × 8 cm openings were cut along the length of the pipe, one on each side. We covered these openings, as well as the bottom of the pipe, with stainless steel mesh attached with rivets and nutrient-free glue. We covered 22 cores each with 1.45 mm, 38 µm, or 5 µm mesh (total of 66 cores) to either allow root and hyphal growth into the core, exclude roots but allow hyphae, or exclude both roots and hyphae, respectively (Johnson, et al. 2001; Langley et al. 2006; Phillips et al. 2012; Moore et al. 2015). We used sand-filled ingrowth bags to estimate mycorrhizal hyphal biomass inside the cores. Previous work demonstrated that sand-filled ingrowth bags exclude non-mycorrhizal fungi (Moore et al. 2015, Wallander et al.

2001). Each ingrowth bag was 8 cm long × 4 cm wide, constructed of 36 µm nylon mesh and nutrient free glue. Each contained 15 cm³ of sand.

On May 12, 2014 we installed cores throughout a 40 m x 40 m study area. We used a random list generator to ensure that the installation order of the three mesh sizes was random across the entire study area. To install cores, we excavated soil using a 5 × 15 cm hammer core (AMS, Inc., American Falls, ID), extracted visible roots from the soil, and then filled each core half way with soil before placing an ingrowth bag inside. The rest of each core was filled and placed in the hole so the mesh was completely submerged. Cores were placed at least 0.5 m away from other cores. Cores were left in the field for 43 d to allow ingrowth of roots and hyphae. After completion of starch addition and field measurements (described below), all cores were collected on June 29, 2014.

Field Measurements and collection

To track the fate of carbon over time we used a 99 atom-% ¹³C labeled starch (Cambridge Isotope Laboratories, Tewksbury, MA, USA. 5 mg of starch, an amount large enough to have a significant label but small enough to not fertilize the soil (Zak and Kling 2006), was mixed into 30 mL of deionized water and injected into 45 cores (15 of each exclusion type). In total we had 45 starch-labeled cores and 21 control cores, in which we only injected deionized water. We injected labels, or water for controls, on June 24, 2014. Cores labeled with ¹³C-labeled starch were sampled for soil respiration and ¹³C-CO₂ on days 1, 2, 3, 4 and 5 after injection. This timeframe ensured we would capture peak microbial respiration of the ¹³C-labeled substrate (Zak and Kling 2006). Soil respiration was measured using a LI-6400XT and soil chamber attachment (LI-COR Instruments, Lincoln, NA, USA). To collect gas samples for ¹³CO₂ analysis, we capped the cores with with a tightly fitting 5 cm diameter PVC cap fitted with a rubber septum. The average rate of soil efflux was used to calculate the length of time needed to allow enough CO₂ build up in the capped cores to detect ¹³C in CO₂. Once the predetermined time was reached (23 minutes for this site), we used a syringe to draw a 15 mL sample of gas from the cap, and injected the sample into a 12 mL Exetainer vacuum vial (Labco Limited, Lampeter, Ceredigion, UK). One gas sample per sampling

day was taken from the cores. At the beginning and end of each sampling day 2 ambient samples were taken to establish background levels of $^{13}\text{CO}_2$. All $^{13}\text{CO}_2$ samples were analyzed at the UC Davis Stable Isotope Facility (Davis, CA USA) using a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE USA). Soil moisture was measured in the field for each sampling day using a Hydrosense TDR probe (Campbell Scientific, Logan, Utah). We removed all cores five days after starch injection, placed their contents in a plastic bag, transported them in a cooler, and stored all soils at 4°C until analyses.

Root, mycorrhizal, and microbial biomass

After collection, we removed soil from the cores and visually inspected soils for roots. We collected and categorized all roots as live or dead, as well as coarse (>2 mm) or fine (<2 mm). We oven-dried the collected root biomass at 60°C for approximately 48 hours before weighing. We converted live, fine root biomass used in analyses from oven-dry weight (mg) per dry weight soil (g) using core volume. To convert this to Mg ha^{-1} we multiplied the mg g^{-1} weight by the core-specific bulk density. We extracted mycorrhizal hyphae using the methods described by Wallander and colleagues (2004). Briefly, we emptied the contents of each hyphal ingrowth bag into a 1 L beaker and added approximately 500 mL of DI water. We stirred the slurry rapidly for 2 minutes on a stir plate. Mycorrhizal hyphae floated to the top of the water and we collected them with forceps. We repeated this process until hyphae no longer floated out, which was approximately 4 times for each sample. We freeze-dried the collected hyphal biomass prior to weighing; freeze-drying is a gentler process of removing water than oven-drying, and it works well for low mass tissues such as hyphae (Olsson & Wilhelmsson 2000). We report mycorrhizal biomass as hyphal biomass (mg) per dry weight sand within the ingrowth bags (g).

We analyzed microbial biomass carbon (MBC) within 48 hours of collection using the chloroform fumigation-extraction method from Vance et al. (1987). We measured

out two replicates of 15 g field-moist soil, sieved to 2 mm. For unfumigated samples, we mixed one replicate with 50 mL of K_2SO_4 then agitated on a reciprocal shaker for 1 h before filtering it through Whatman #1 filter paper. For fumigated samples we placed the second 15 g replicate in a glass desiccator with 30 mL of chloroform, evacuated the desiccator to boil the chloroform, sealed it, then left the soils for 5 days at room temperature. Fumigated samples were extracted in the same manner as non-fumigated samples. All samples were stored at $-4^{\circ}C$ until analysis. We measured microbial biomass carbon on a total organic carbon analyzer (TOC-V CPH Total Organic Carbon Analyzer, Shimadzu Scientific Instruments, Columbia, MD). Microbial biomass is the final (fumigated) sample minus the initial (non-fumigated) sample multiplied by a correction factor of 0.38 (Voroney, Winter & Beyaert 1993) and is presented in units of $mg\ C\ g^{-1}$ dry soil (Voroney et al. 1993).

We analyzed the potential carbon and nutrient enzyme activity of our soils using methods described by Bell and colleagues (2013) within 48 h of collection. Briefly, we measured out 2.75 g of field moist soils, sieved to 2 mm, and mixed it with 91 mL of 50 mM sodium acetate buffer with a pH of 5 using an immersion blender. We pipetted 800 μL of soil slurry into a column on a deep (2 mL) 96-well plate that contained increasing concentrations of non-linked MUB substrate in rows A-G, ranging from 0 -100 μM (MUB standard plate). We then pipetted another 800 μL of the soil slurry into a column on an empty deep 96-well plate (sample plate). We continued this process until all soils had been blended and plated. We then added 200 μL of 1 of 5 different 4-methylumbelliferone-linked (MUB-linked) substrates into the rows of the sample plate. Each substrate has its own row; substrates consisted of 4-MUB- β -D-glucoside (β -gluc) and N-acetyl- β -D-glucosaminide (NAG). We sealed each plate with a plate mat, agitated vigorously by hand, then allowed them to incubate in the dark at room temperature for 3 h. After incubation we transferred 250 μL of the slurry from each cell to a black 96-well plate. Using a fluorimeter/spectrophotometer (Synergy HT, Biotek Inc, Winooski, VT) we measured fluorescence of hydrolytic enzymes at an excitation of 365 nm and an emission of 450 nm. Potential enzymatic activity is expressed in units of $nmol\ h^{-1}\ g^{-1}$ dry soil.

To statistically test for relationships among mycorrhizal roots and hyphae and their abiotic environment we analyzed bivariate correlations. Data were adjusted to meet assumptions of normality prior to statistical tests (D'Agostino 1970) using either log or square root transformations. We tested the correlation between root biomass and mycorrhizal biomass and between each biomass measure and pH and gravimetric water content (N = 66). To test how roots and mycorrhizal fungi influenced microbial metabolism of a labile substrate, we analyzed the relationship between ^{13}C -labeled starch gas samples and each biomass group using linear regression. Only cores where starch was added to the soil was analyzed (n = 15). While evidence from similar studies suggested mesh size was not correlated with mycorrhizal root or hyphal biomass (Moore et al. 2015), we tested for any effects of mesh size on biomass to be certain. Because earlier studies showed starch addition did not affect these measurements (Moore et al. 2015), we combined the water-added and starch-added cores and only analyzed the effect of different mesh sizes (n = 22). All analyses were performed in R (R Core Team 2014).

We developed three alternate structural equation models (SEMs) to address the hypothesis that root and mycorrhizal biomass affect microbial biomass and activity (Grace et al. 2012) using the R package lavaan (Rosseel 2012). We tested our SEMs using observed data collected in our field study. One SEM tested whether microbial biomass was affected by root and MBC, and all microbial activity indicators (β -glucosidase, NAGase, and soil respiration) were driven by MBC and correlated with one another. Thus, there were no direct pathways from root or hyphal biomass to microbial activity that were not mediated by MBC. A second SEM contained the same relationships as the previously described SEM, but with added direct pathways of roots on β -glucosidase and hyphae on NAGase activity. We added these pathways because their model index statistic was $X^2 > 3.84$ (df = 1), indicating a high chance of these pathways explaining patterns within our data set (Grace et al. 2012). A third model was a fully-saturated null model that contained all possible effects. Within each of the three alternate SEMs, we defined two “groups” based on hyphal presence for each model (using the “group” parameter within the “sem” function) because many of our samples

contained no measured hyphal biomass. Thus, in the hyphae-absent group there were no pathways that included hyphae, while in the hyphae present group pathways linked hyphal biomass to endogenous variables.

We tested for goodness-of-fit between each model and our empirical data set using a χ^2 test. Good model fit is indicated when $0 \leq \chi^2 \leq 2$ and $0.05 \leq P \leq 1.00$. We compared the goodness-of-fit among our models and reported the model that best fit our empirical data set. Model selection was based on AICc scores and models within two AICc points were assumed to have similar data-model fit. The best model was the one with the lowest AICc score. AICc was chosen rather than AIC due to our small (<200) sample size (Burnham & Anderson 2004). For the best fit model, we reported standardized path coefficients, which have standardized units making comparison among pathways within a model possible. We also reported total effects that each biomass variable has on microbial activity indicators. The total effect was calculated as the sum of all direct and indirect effects between two variables (Grace 2006). If a pathway between two variables contained an intermediate variable, then all coefficients along that pathway were multiplied. If there were multiple pathways between two variables, the sum of all of the pathways was considered the total indirect effect.

Results

Our experimental ingrowth core design successfully manipulated root and hyphal biomass enabling us to examine their effects on microbial activity. Root biomass ranged from $7.45\text{E-}4$ to 31.12 mg g^{-1} and hyphal biomass ranged from 0 to 87.6 mg kg^{-1} . Mesh size and ^{13}C -starch addition was not correlated with root biomass ($P = 0.44$, $F = 0.84$; $P = 0.45$, $F = 0.31$, respectively; Table 3.1) or hyphal biomass ($P = 0.59$, $F = 0.54$; $P = 0.45$, $F = 0.57$). Similarly, soil respiration was unrelated to mesh size across 5 days ($P = 0.07$, $F = 2.79$; Table 3.1) or starch addition on total soil respiration ($P = 0.59$, $F = 0.06$; Table 3.1). Soil respiration was highest 4 days after starch addition treatments ($P < 0.001$, $F = 11.62$) likely due to a rainfall event.

Root biomass was not correlated with mycorrhizal biomass ($P = 0.87$, $R^2 = <0.01$). Soil pH was not correlated with root biomass ($P = 0.25$, $R^2 < 0.01$) or

mycorrhizal biomass ($P = 0.50$, $R^2 < 0.01$). Soil GWC ranged from 24 to 83% with a median of 33%. GWC increased by 2% per mg of root biomass ($P < 0.01$, $R^2 = 0.28$), but it was not related to mycorrhizal biomass ($P = 0.24$, $R^2 < 0.01$).

There was a mid-biomass effect of roots on enzyme activities. Carbon-degrading enzyme activity, indicated by β -glucosidase activity, was highest between 4 – 9 mg g⁻¹ root biomass ($P < 0.01$, $R^2 = 0.12$ Fig. 3.1a), but was not related to mycorrhizal biomass ($P = 0.65$, $R^2 < 0.01$). Nutrient-acquiring enzyme activity, indicated by NAGase activity, was lowest between 4 – 9 mg g⁻¹ root biomass ($P < 0.01$, $R^2 = 0.12$ Fig. 3.1b), but was not correlated with hyphal biomass ($P = 0.76$, $R^2 < 0.01$). Metabolism of ¹³C-starch did not vary with root ($P = 0.35$, $R^2 < 0.01$) or mycorrhizal biomass ($P = 0.53$, $R^2 < 0.01$, Fig 3.1c).

The best fit structural equation model included a combination of indirect and direct effects of roots and hyphae on microbial activity ($P = 0.27$, test statistic = 9.96, df = 8; $P > 0.05$ indicates strong data-model fit; Fig 3.2). The fit of the best model (AICc = 748.68) was stronger than a simpler model that lacked direct effects of roots and hyphae on microbial activity (AICc = 759.62) or a fully saturated null model (AICc = 784.95). In the model group with hyphal presence, the strongest total effects were of roots on MBC (standardized path coefficient = 0.52) and of roots on β -glucosidase (standardized path coefficient (SPC) = 0.53). Microbial biomass C had a stronger effect on NAGase (SPC = 0.26) than on β -glucosidase (SPC = -0.03). The effect of hyphae on NAGase (SPC = 0.26) was greater than the effect of hyphae on MBC (SPC < 0.01), β -glucosidase (SPC < -0.01), or soil respiration (SPC < 0.01). Soil respiration was more strongly affected by MBC (SPC = 0.26) than by roots (SPC = 0.14) or hyphae (SPC < 0.01). Microbial biomass was more strongly affected by roots (SPC = 0.52) than by hyphae (SPC < 0.01). The effects captured by the best fit model explained 33% of the variation in MBC, 26% of variation in β -glucosidase, 34% of variation in NAGase, and 30% of variation in soil respiration.

Discussion

Roots and mycorrhizal fungi affect microbial activity in distinctly different ways as shown by our best-fit SEM model (Fig. 2). We anticipated mycorrhizal modification of root influences on microbes, and thus an interactive term between roots and mycorrhizal fungi. However, the model that best fit our empirical data lacked an interaction term between roots and mycorrhizal fungi. While some researchers propose a holistic perspective of plant-mycorrhizal effects on ecosystem processes (e.g. Johnson et al. 2013, Phillips et al. 2013), we suggest further investigation into isolated effects of roots and mycorrhizal fungi on microbial decomposition activity and other ecosystem processes. The best-fit model suggested that roots increased C-degrading enzyme activity while mycorrhizal fungi increased N-acquiring enzyme activity.

Our SEM model suggested a strong causal link between roots and C-degrading activity of free-living soil microbes. β -glucosidase, a C-degrading enzyme involved in cellulose decomposition, was more strongly stimulated by root activity directly than indirectly via microbial biomass. Others have found that roots stimulate enzyme activity of microbes (Brzostek et al. 2015). Roots can directly affect microbial activity if enzyme activity rates change with no change in microbial biomass such as when microbial C allocation shifts or if there is a shift in the microbial community (de Graaff et al. 2010). DeAngelis et al. (2009) found that actively growing roots affected soil bacterial communities by changing abundances of about 7% of the soil bacterial taxa. The bacterial community also responds to root sugars and exuded organic acids (Shi et al. 2011), which change the pH of soil and thus affect microbial community structure (Fierer and Jackson 2006). We found the direct effect of roots on β -glucosidase activity was 95% of the total effect, suggesting there is a strong influence of roots on soil enzyme activity, however mechanisms behind this pattern need to be further explored.

Roots did not directly affect the activity of the nutrient-acquiring enzyme NAGase, which is used to acquire N from chitin in soil. This result differed from our hypothesis that roots would have universal effects on all enzymes involved in decomposition activity. Because soil organic matter contains C and N, we thought C- and N-degrading enzymes would be equally impacted by roots. Surprisingly, the total effect of roots on

NAGase activity was one-third of their total effect on β -glucosidase activity. This is opposite of other studies showing that immobilization of N was increased in presence of roots (Holz et al. 2015); the authors hypothesized this was due to stimulated microbial activity in presence of roots.

Unlike roots, mycorrhizal biomass directly affected NAGase activity as shown in our SEM model (Fig. 2a). Again, this result was counterintuitive because we hypothesized that suppression of microbial growth by mycorrhizae would mediate any effect of mycorrhizae on soil enzyme activity (i.e., that indirect effects would be stronger than direct effects). The direct effect of mycorrhizal biomass on NAGase activity accounted for 99% of the total effect of mycorrhizae on NAGase. Similar to our finding, Kluber et al. (2011) found increased NAGase activity in mycorrhizal-dominated fungal mats in a Douglas-fir forest. The increased NAGase activity was attributed to mycorrhizal modification of the soil microbial community, but that mechanism was not verified. We found the causal pathway between mycorrhizal biomass and microbial biomass was non-significant, and that the direct effect of mycorrhizae on NAGase activity was equal in magnitude to the direct effect of microbes on NAGase activity. The direct effect of mycorrhizal biomass on NAGase activity can be interpreted in at least two ways. Mycorrhizal fungi could be producing NAGase themselves, in which it would make sense that more mycorrhizal biomass leads to more NAGase production. In a lab culture study, ectomycorrhizal fungi secreted NAGase to break down chitin, but their NAGase activity was an order of magnitude lower than non-mycorrhizal soil fungi (Hodge et al. 1995). The amount of NAGase released by mycorrhizal fungi in natural settings is likely even lower than that.

Another potential mechanism for direct effects of mycorrhizal fungi on NAGase is mycorrhizal fungal selection for a microbial community with increased NAGase production. Support for this idea was found in a dual-chamber greenhouse study, where AM fungi were associated with a soil prokaryotic community unique from the community found in mycorrhizae-absent soil (Nuccio et al. 2013). Bacteria that more efficiently degrade organic matter increased in abundance with mycorrhizal presence. This community change altered decomposition rates of ^{15}N -labeled root litter, which in turn

decreased foliar C:N by 30% (Nuccio et al. 2013). Mycorrhizae can select for microbes by altering the soil environment through production of oxidative enzymes and competition for other resources. Selecting for a community with heightened NAGase production would be beneficial for mycorrhizae as it would increase the availability of N in the soil, which mycorrhizae exchange with plant tissues.

Metabolism of ^{13}C -labeled starch was highest where root biomass was zero and hyphal biomass was low, however the pattern was non-significant in a linear regression (Fig. 1c). A potential cause of increased microbial break down of ^{13}C -starch, a form of labile C, where roots are not present is that absence of roots could amplify C-limitation of microbial growth. Another peak in ^{13}C -starch metabolism was detected where root biomass was high and hyphal biomass was low. This matched the pattern we found for β -glucosidase activity, likely because microbes were using root-derived C to produce β -glucosidase enzymes in order to break down cellulose in soil. Metabolism of ^{13}C -starch was lowest where mycorrhizal biomass was high. This may be explained by mycorrhizal influences on microbial community structure and function, suppressing C-degraders while stimulating N-acquirers, but this mechanism remains to be tested. The patterns we found between microbial activity, roots and mycorrhizal biomass corroborate a simulation model where mycorrhizal fungi suppressed C-degrading activity of soil microbes especially in N-limited scenarios (Orwin et al. 2011). Mycorrhizae may foster an N-acquiring community rather than a C-degrading community (Nuccio et al. 2013) because their growth is limited by N and not C.

Mycorrhizal biomass was not correlated with root biomass and has a patchy distribution in soil. More than half the cores (56%) contained zero mycorrhizal biomass while all cores contained at least some root biomass. A common assumption is that mycorrhizal biomass increases with root biomass as mycorrhizae depend on roots for a supply of C. However, in boreal forest ecosystems, some ectomycorrhizae are capable of acquiring C by decomposing organic matter (Read et al. 2004), which could explain the disconnected relationship with root biomass. Many samples had low mycorrhizal biomass overall, and a few samples had very high mycorrhizal biomass. Of the soil characteristics we measured, we found none correlated with mycorrhizal colonization in

our cores. Mycorrhizal biomass was not correlated with soil moisture or pH, which are thought to shape soil microbial communities (Fierer and Jackson 2006).

Our results provide empirical evidence for future ecosystem model development. Ecosystem models will likely improve their predictive power after explicitly representing microbial activity (Treseder et al. 2012, Schimel 2013). The common way of doing this is by using Michaelis-Menten functions to describe extra-cellular microbial enzyme activity, which typically describe a saturating curve that is limited by microbial biomass (Lawrence et al. 2009, Wang et al. 2013). We have shown here that roots and mycorrhizal fungi can alter rates of enzyme activity without changing microbial biomass. Furthermore, the effect of mycorrhizae on microbial biomass was non-significant while the effect of mycorrhizae on NAGase activity was moderately strong. Taken together, this study demonstrates that implicit assumptions of root and mycorrhizal effects on enzyme-driven decomposition of organic matter are unrealistic, and explicit consideration may increase ecosystem model performance.

Overall, we demonstrated that roots and mycorrhizal fungi have different effects on microbial activity in soil. Roots had a direct effect on C-degrading enzyme activity while mycorrhizae had a direct effect on N-acquiring enzyme activity. These effects were independent of influences of roots and mycorrhizal fungi on microbial biomass. We suspect this is due to roots and mycorrhizae causing shifts the microbial community, which has downstream effects on decomposition of organic matter in soil.

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Appendix: Figures

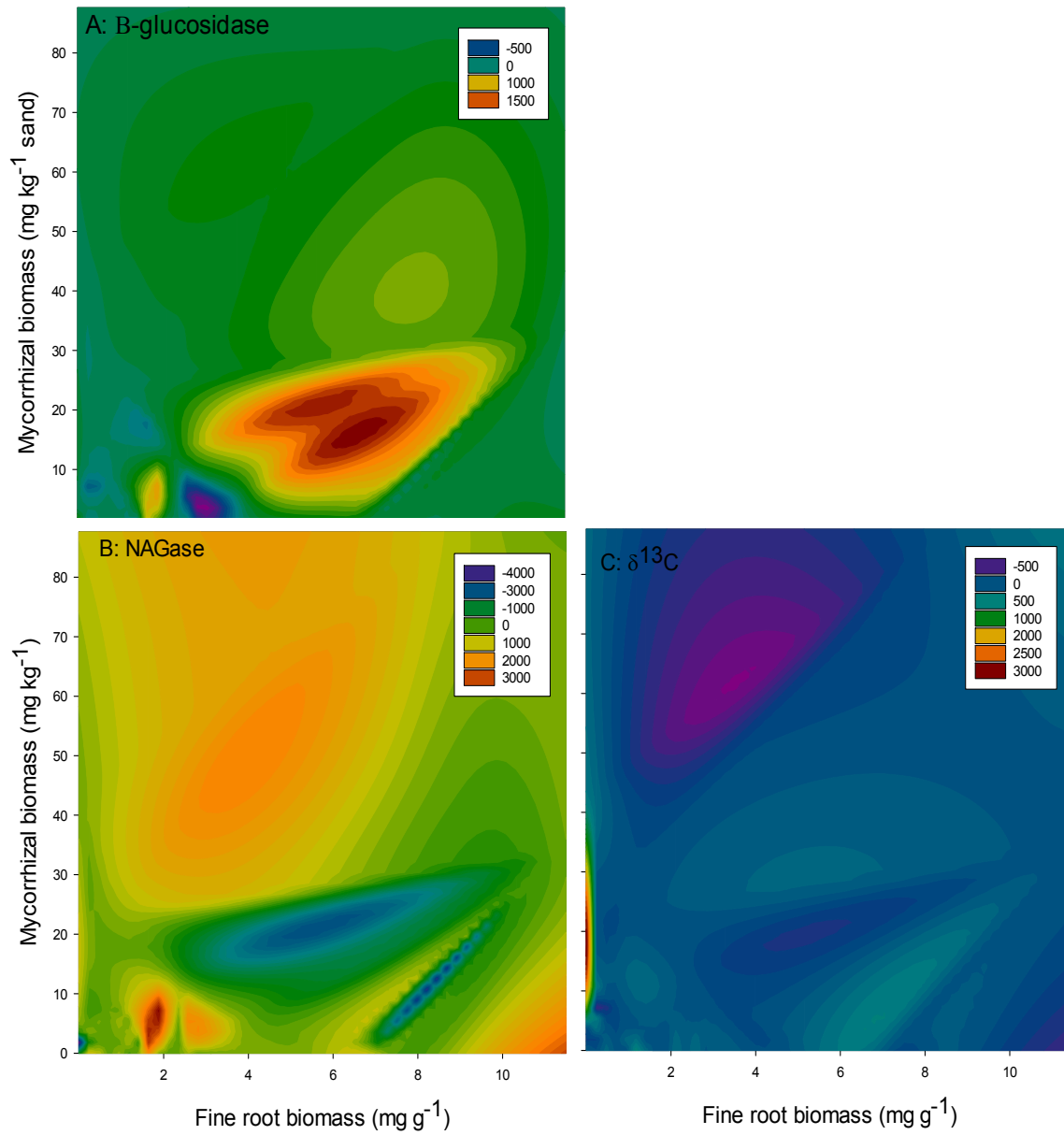


Figure 3.1. Root and mycorrhizal effects on enzyme activities and metabolism of ^{13}C -starch. (A) β -glucosidase activity ($\text{nmol g}^{-1} \text{h}^{-1}$); (B) NAGase activity ($\text{nmol g}^{-1} \text{h}^{-1}$); (C) $\delta^{13}\text{C}$ (‰) only in cores where ^{13}C -starch was added to soil. Red colors indicate higher activity while blue colors indicate lower activity ($N = 66$).

Figure 3.2. Path diagrams representing best fit structural equation models subset into two groups, with (A) and without (B) mycorrhizal biomass. Exogenous variables appear in boxes while endogenous variables appear in ovals. Single-headed arrows represent causal effects, and double-headed arrows represent correlations. Black solid lines show positive effects and dashed lines show negative effects. Gray lines are non-significant effects. Standardized path coefficients are noted on each path. Standardized correlation coefficients are shown for each endogenous variable. Roots, fine root biomass; mycorr., mycorrhizal hyphal biomass; MBC, microbial biomass carbon; Bgluc, β - glucosidase potential enzyme activity; resp., soil respiration; NAG, potential N-acetylglucosaminidase enzyme activity.

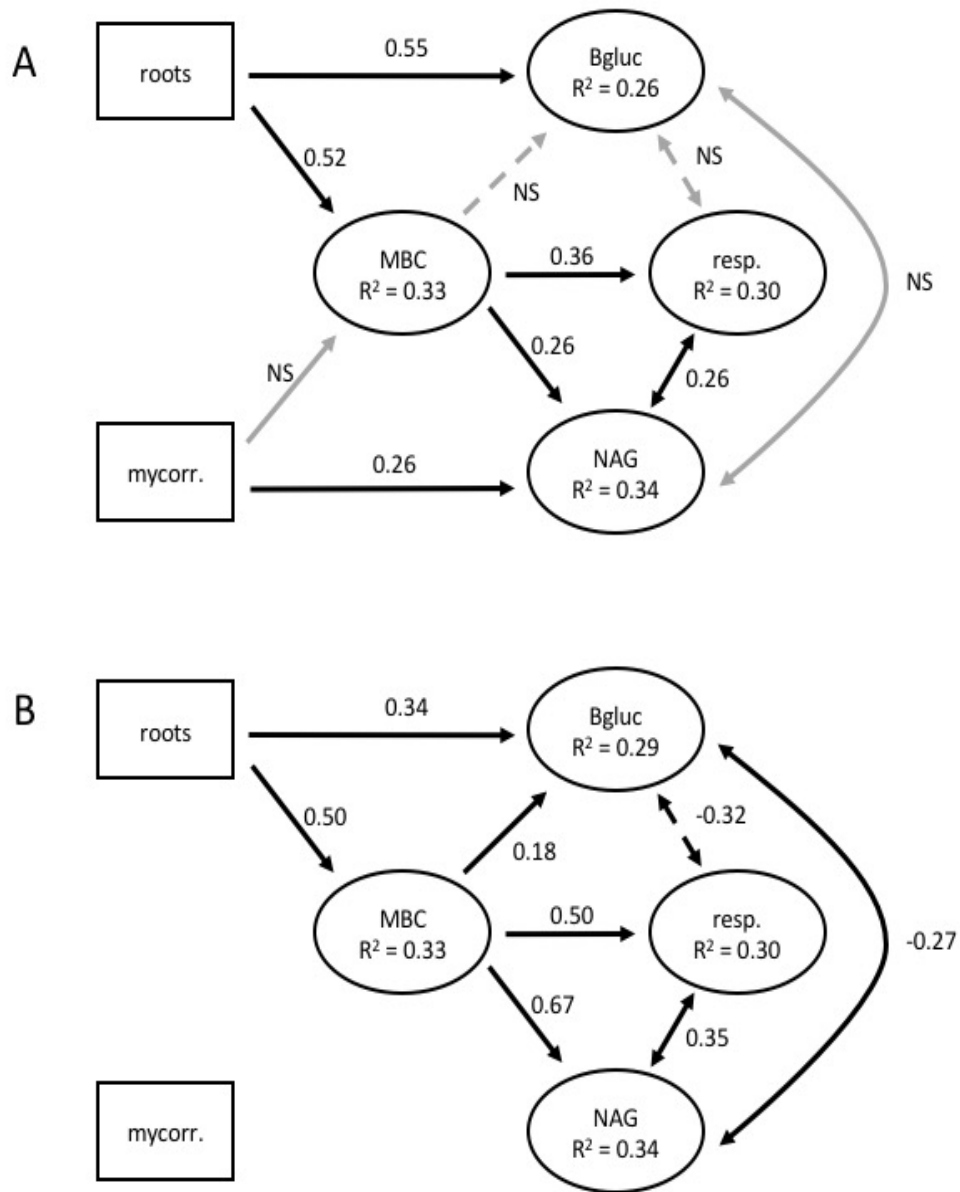


Figure 3.2. Continued.

Appendix: Tables

Table 3.1. Mean ($\pm$ standard error) of environmental variables, biomasses, and indicators of microbial activity in each exclusion and group. RM, high root and high mycorrhizal biomass; M, low root and high mycorrhizal biomass; excl., low root and low mycorrhizal biomass; ^{13}C , labeled-starch addition; W, water addition; GWC, gravimetric water content (%); root, root biomass (mg g^{-1}); hyphal, hyphal biomass (mg kg^{-1}); MBC, microbial biomass C (mg C kg^{-1}); soil resp., soil respiration ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); β -gluc, β -glucosidase potential activity ($\text{nmol g}^{-1} \text{ h}^{-1}$); NAG, N-acetylglucosaminadase potential activity ($\text{nmol g}^{-1} \text{ h}^{-1}$). See Methods for details on exclusion and ^{13}C -label groups.

Parameter group	Parameter	RM + ^{13}C	M + ^{13}C	excl. + ^{13}C	RM + W	M + W	excl. + W
<i>Environmental</i>	GWC	40.0 (13.6)	33.9 (9.6)	34.1 (3.9)	35.2 (7.0)	39.0 (20.0)	34.0 (10.5)
	soil C:N	22.5 (22.2)	21.4 (14.8)	23.7 (14.9)	23.8 (23.7)	25.3 (16.6)	15.9 (1.7)
	soil pH	5.1 (0.5)	5.1 (0.4)	5.0 (0.3)	4.9 (0.6)	4.8 (0.4)	4.9 (0.7)
<i>Biomass</i>	root	3.4 (4.1)	1.4 (2.1)	1.2 (1.6)	1.7 (2.3)	5.0 (11.6)	1.3 (1.4)
	hyphal	9.0 (23.5)	4.7 (7.0)	4.9 (11.6)	4.2 (10.3)	5.0 (6.7)	1.0 (2.0)
	MBC	904.5 (858.0)	586.1 (540.5)	423.4 (184.9)	505.9 (396.0)	441.4 (211.0)	415.1 (310.7)
<i>Activity</i>	soil resp.	4.7 (1.7)	4.2 (2.1)	4.0 (0.9)	5.0 (2.1)	4.2 (0.9)	3.4 (0.5)
	β -gluc	175.1 (104.7)	163.5 (70.2)	142.8 (50.8)	152.1 (69.4)	154.0 (59.1)	160.8 (88.4)
	NAG	648.7 (352.3)	395.1 (143.3)	438.5 (120.3)	483.5 (260.9)	347.5 (92.4)	399.6 (157.8)

Conclusions

Carbon is assimilated by plants, transferred to mycorrhizal fungi, and decomposed by free-living soil microbes. Thus, each of these soil community members is integral to ecosystem C dynamics. Their influence on C is extended via their interactions with one another. For example, mycorrhizal roots transfer more C belowground than non-mycorrhizal roots, and soil microbes decompose more organic matter in presence of roots than in absence of roots. However, there are few studies on mycorrhizal interactions with roots and soil organisms in light of soil C accrual and release. In my doctoral research, I examined interactions among plants, mycorrhizal fungi, and soil microbes in order to better understand the role of mycorrhizal fungi in soil C dynamics by combining models with experiments. My work is the first to demonstrate that the direct role of mycorrhizal fungi on organic matter decomposition was slight compared to decomposition by free-living microbes. I tested for additive effects of roots and mycorrhizal fungi, but I found the relationship was more complex than expected. Mycorrhizal roots increased soil enzyme activity rates, but when roots were removed enzyme activity was lower. I suggested this was driven by nutrient demand of mycorrhizal fungi. Finally, I examined microbial decomposition of carbon and nutrient substrates across a range of root and mycorrhizal influence, including their direct and indirect effects. I found that roots directly increased C-degrading enzyme activity while mycorrhizal fungi increased N-acquiring enzyme activity in soil. My dissertation advanced the field of ecosystem ecology by revealing how roots and mycorrhizal fungi diverge in their effects on free-living microbial function, and the implications plant-mycorrhizae-microbe interactions have on soil C dynamics.

Future directions

Closely linking model and experimental work has improved our ability to predict ecosystem responses to climate change, yet this approach is uncommon especially when considering belowground communities and function (Luo et al. 2011, Treseder et al. 2012, Todd-Brown et al. 2012). My work addressed this knowledge gap by modeling and experimentally manipulating belowground interactions. Plant-soil community responses to fluctuations in climate are unpredictable (Classen et al. 2015). Thus, experimentally testing how these communities and their interactions respond to climatic

changes is necessary if scientists are to better predict how climate change will alter ecosystems (Wall et al. 2008, Luo et al. 2011). Furthermore, understanding biological mechanisms behind patterns of carbon pools and fluxes is pertinent to the improvement of currently used carbon and climate models.

My research demonstrated that interactions within the rhizosphere can affect ecosystem processes, however, these ecological interactions between mycorrhizae and the soil microbial community could be contingent upon climate and biotic pressures like competition (Van der Putten 2009). The stress-gradient hypothesis (Callaway and Walker 1997, Chakraborty et al. 2012) predicts more facilitative interactions in physiologically stressful environments and more competitive interactions in moderate environments. This hypothesis has been tested in aboveground plant communities, showing fitness benefits of facultative neighbors in stressful environments.

Testing the stress-gradient hypothesis in a belowground context could reveal how climate alters belowground interactions. Drought stress and resource availability change along climate gradients and directly influences the carbon allocated belowground and specifically to mycorrhizal fungi. When drought stress is alleviated, increased physiological rates are manifest as increases in total biomass and decomposition activity (Hawkes et al. 2011). On the contrary, as temperature increases, microbial carbon use efficiency is reduced leading to lower microbial biomass (Allison et al. 2010). The combined effects of moisture and temperature stress on microbial activity further affect ecosystem processes like carbon cycling (Allison and Treseder 2008, Djukic et al. 2013).

Rates of ecosystem processes, like C and N transformations, are contingent upon microbial community diversity. Microbial communities are functionally redundant for some ecosystem processes like total decomposition (Nielsen et al. 2011). However, decomposition of different C substrates ranging from simple to complex is higher (Miki et al. 2014) and denitrification is higher (Phillippot et al. 2013) in ecosystems with diverse microbial communities compared to those with few microbial taxa. Similarly, N fixation increases where diversity of N-fixing bacteria is high, leading to “hot spots” across the landscape (Reed et al. 2010). Interactions among microbes can further lead

to synergisms and affect ecosystem functioning so that the whole is greater than the sum of its parts (Mikola and Setälä 1998). As microbes compete or are subject to different predation rates, functions such as C and N mineralization can be altered in ways that are not fully predicted by total diversity (Mikola and Setälä 1998). Microbial communities with similar taxa can function differently across a range of environments (Zak and Cling 2006). Understanding the factors controlling the strength of the link between microbial community structure and ecosystem function is important for predicting ecosystem processes in a changing world.

Once we understand how climate alters belowground interactions and the context-dependency of microbial community structure-function relationships, we can create carbon cycle models that are more representative of natural systems. Terrestrial ecosystem models (Jenkinson *et al.* 1991, Parton *et al.* 1998) and Earth System Models (ESMs) (Oleson *et al.* 2010) implicitly account for soil microbial activity by assuming a constant rate (i.e., 1st order function) of organic matter decomposition. My research demonstrated non-linear effects of root-mycorrhizae-microbe interactions on carbon and nutrient fluxes. Others have explored biotic effects on decomposition through inter-guild interactions (e.g. Moorhead & Sinsabaugh 2006), plant-microbe interactions (e.g. Orwin *et al.* 2011), and microbial trait trade-offs (e.g. Allison 2012). Whether non-linear microbial activity alters predictions of soil C stocks and fluxes at larger spatial and longer time scales remains a knowledge gap (Schimel 2013). Future research will need to compare predictions of first-order and mechanistic models across a range of parameter values representing various ecosystem, soil, and climate combinations. After identifying the parameter combinations where models diverge in their predictions, we can design experiments that test the models' underlying assumptions of microbial effects, such as whether ecological interactions at the micro scale are needed to capture element transformations at the ecosystem or global scale.

Vita

Jessica Moore's academic career began at Eastern Washington University where she earned a dual Bachelor of Science degree in Biology and Environmental Science. She focused her studies on ecology, botany, and mycology. With support and funding from the Ronald E. McNair Scholar's Program, she completed an independent research project with mentorship from plant ecologist Dr. Rebecca Brown. Jessica assessed biotic and geologic factors influencing plant invasion in a national wildlife refuge. In addition to research, she spent time working in the Biology Department greenhouses and mentored peers through the Program Leading to Undergraduate Success. Jessica's experiences at Eastern Washington University prepared her for doctoral research at University of Tennessee.

Jessica's interest in fungi and their effects on ecosystem processes sparked the research questions described within this dissertation. She began by exploring direct effects of mycorrhizal fungi on carbon fluxes in soil using a mechanistic model she developed. She applied theory developed for free-living microbes to mycorrhizal fungi, marking this study as unique from other ecosystem models that omit mycorrhizal activity. Direct effects of mycorrhizal fungi naturally led her to be curious about their indirect effects via other soil microbes. Jessica established field sites in eastern Tennessee and northern Minnesota. She manipulated roots and mycorrhizae in soil to explore how microbes function with and without pressures or assistance from mycorrhizal fungi. These basic ecological interactions have only recently been investigated as the perspective of mycorrhizal fungi represented in the literature shifts from mycorrhizae as plant symbionts to mycorrhizae as soil microbes. Jessica's work has contributed to this shift in perspective and it serves as a critical step toward considering the role of mycorrhizal fungi within microbial communities and as agents of ecosystem processes.

In addition to her dissertation research, Jessica has collaborated with other scientists. She worked closely with J. Jiang to incorporate plant-mycorrhizal interactions into resource ratio theory. She collaborated with evolutionary ecologists to examine how communities of soil arthropods varied with tree evolutionary relatedness and whether

that relationship helped explain ecosystem processes. With a group of plant ecologists, she investigated how fire-regimes alter plant-pollinator interactions. She co-authored a perspective article on how climate change can directly and indirectly alter soil microbial communities. She is also scaling up her plant-microbe ideas to a global scale in a co-authored perspective article on future directions of Earth system model development. In sum, Jessica Moore's collaborations broaden her research scope and have enabled her to pursue her general interests in ecological interactions and processes.