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SPECIAL FEATURE – STANDARD PAPER

WHETHER IN LIFE OR IN DEATH: FRESH PERSPECTIVES ON HOW PLANTS AFFECT BIOGEOCHEMICAL CYCLING

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

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Summary

1. 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.

2. 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 mycorrhizas are isolated from roots, they may limit soil carbon decomposition by competing with free-living decomposers for resources.

3. 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 in respiration and 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.

4. 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-labelled starch was highest when root biomass was high and mycorrhizal biomass was low. These results suggest that mycorrhizas 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.

5. *Synthesis*. Our results suggest that the activity of enzymes involved in organic matter decomposition was contingent upon root–mycorrhizal–microbial interactions. 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.

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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, Bardgett & van Straalen 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, Malhi & Morecroft 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, Kandeler & Ruggiero 2002). After breaking apart organic matter, the microbial community mineralizes it, which provides plants with nutrients that are essential for them to grow. These degradation and mineralization processes release large amounts of carbon from the soil back to the atmosphere as CO_2 (Fenn, Malhi & Morecroft 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 variety of ways (Hogberg, Hogberg & Myrold 2007; Phillips *et al.* 2012; Clemmensen *et al.* 2013). For example, roots and mycorrhizas 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 years) organic matter (Kuzyakov 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, Mariotti & Abbadie 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 mycorrhizas can facilitate or compete with other soil microbes (Frey-Klett *et al.* 2011). Mycorrhizal fungi can facilitate free-living microbial communities 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 mycorrhizas 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 out-compete 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 among 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, mycorrhizas and free-living microbial communities 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 (Fig. 1). 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 probably 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 that 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-labelled substrate as a tracer to track the source of carbon respired by the soil microbial community. To explore whether 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 among roots, mycorrhizal fungi and free-living microbial communities 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 year^{-1}. Bulk density of the B-horizon was 1.4–1.5 g cm^{-3} with a pH value 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 ^{13}C-labelled starch addition. We constructed 30 soil mesocosms using 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 or 5 μm stainless steel mesh to allow root and mycorrhizal access, exclude roots or exclude roots and mycorrhizas, 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 15 May 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, USA) 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 the high-clay soil. Prior to establishing the experiment, we tested several ratios of soil–sand mixtures and determined a 1 : 1 ratio of 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 where 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 of autoclaved quartz sand approximately 5 cm below the soil surface. Mesocosms were stabilized in the field for 16 weeks to allow root and mycorrhizal colonization.

On 9 September 2013, we mixed 5 mg of 99 atom-% ^{13}C-labelled 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 4 days, on 13 September 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 min, we extracted four 15-mL gas samples over a period of 10 min from each mesocosm 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 $^{13}CO_2$ samples collected from each core and the average $^{13}CO_2$ background measurement were used to construct a Keeling plot. The Keeling plot enabled us to calculate the $\partial^{13}C$ 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 with a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE, USA).

On 13 September 2013, after the $^{13}CO_2$ samples were collected and respiration was measured, the mesocosms were harvested and kept cool until the soils were processed in the laboratory within 48 h. When appropriate, data are shown on an oven-dry mass basis. We determined gravimetric water content (GWC) for each mesocosm by oven drying approximately 15 g of field-moist soil at 105 °C for 48 h (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 to glucose, while oxidative enzymes degrade aromatic compounds. Enzymes were measured on field-moist soil within 48 h of collection using standard methods (see Saiya-Cork, Sinsabaugh & Zak 2002). We homogenized 1 g of field-moist soil with 125 mL of 0.5 M sodium acetate buffer (buffer, pH 5) for 2 min with an immersion blender. Soil was mixed with 50 μL of methylumbelliferyl (MUB)-linked B-glucoside, MUB-linked cellobioside, 3,4 dihydroxyphenylalanine (L-DOPA) or 10 μL of hydrogen peroxide as substrate 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, non-linked MUB, a negative control of buffer, 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, soil slurry and buffer and two replicates of soil slurry and L-DOPA substrate. We incubated the 96-well plates at room temperature for 2 h (hydrolytic enzymes) or 24 h (oxidative enzymes) in the dark before analysing them on a flourimeter/spectrophotometer (Synergy HT; Biotek Inc, Winooski, VT, USA). 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 nmol h^{-1} g^{-1} dry soil.

We analysed microbial biomass carbon (MBC) using a chloroform fumigation extraction described by Vance, Brookes & 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, USA) for 1 h. We filtered the extraction through Whatman # 1 filter paper previously leached with 100 mL of 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 MBC on a total organic carbon analyser (TOC-V CPH Total Organic Carbon Analyzer; Shimadzu Scientific Instruments, Columbia, MD, USA) 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 2 weeks of collection. Live fine and coarse roots were removed, dried at 60 °C for approximately 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 milligram of hyphal biomass per gram of sand contained in the in-growth bag (Wallander, Goransson & Rosengren 2004).

We identified mycorrhizal taxa by sequencing hyphae collected in five 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, USA). DNA was diluted 1 : 10 with autoclaved DI water prior to being amplified by polymerase chain reaction (PCR) using goTAQ polymerase (Promega, Madison, WI, USA). To target fungal DNA, we used ITS-1F and ITS-4 forward and reverse primers. The PCR program 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 cleanup kit (QIAquick PCR Purification kit; Qiagen, Valencia, CA, USA). 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, USA). Sequences were trimmed in SEQUENCHER 5.3 software (Gene Codes Corporation, Ann Arbor, MI, USA) 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 data base. 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, Elliott & Paustian 2000). We weighed 10 g of air-dried 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.) for 18 h. 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 > 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 analysed for total carbon content at UC Davis using an Elementar Vario EL Cube or Micro Cube elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany).

STATISTICAL ANALYSES

First, we tested to see whether our experimental design worked to reduce root and mycorrhizal biomass and whether our experimental treatment (mesh size) altered soil properties such as soil moisture content. We used a one-way ANOVA to test whether our treatments (+R+M, +M−R, −R−M) effectively excluded roots and mycorrhizas and whether the different mesh sizes influenced soil moisture using R (Zar 1999; R Development Core Team 2011). Our experimental treatments worked to reduce root and mycorrhizal presence; however, the amount that roots and mycorrhizas were reduced varied significantly across our exclusion treatments. Next, we wanted to test whether 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 have to analyse these sets of cores as unique treatments. Using *t*-tests, we compared microbial activity (enzymes and respiration) and soil carbon pools [microbial biomass, particular organic carbon (POC), mineral-associated organic carbon (MOC), dissolved organic carbon (DOC)] in mesocosms where starch was added with mesocosms that only received water (controls). Data were tested for normality using the Shapiro–Wilk test, 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 (GLMs) to examine the effect of root biomass, mycorrhizal biomass and the interaction between root and mycorrhizal biomass on soil carbon processes (Zar 1999). Our ^{13}C-labelled 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. GLMs with a poison distribution were fit separately for our response variables: GWC, 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 $^{13}CO_2$ in mesocosms.

MODEL SIMULATIONS

To explore how our experimental treatments might impact soil carbon dynamics over longer time periods (30 years), 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 extracellular enzymes produced by microbes. It integrates measurable pools of physically defined POC, MOC and 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 (Allison, Wallenstein &

Bradford 2010; Wang, Post & Mayes 2013; Wieder, Bonan & Allison 2013). Of these parameters, enzyme activity rates would most clearly affect V_{max}. Using the model, we explored whether 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 (Wieder, Bonan & Allison 2013; Sulman *et al.* 2014) 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 1.4–1.5 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 modelling is necessary to extrapolate to longer time-scales (30 years). 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, MA, USA). To determine the role of roots and mycorrhizal fungi in 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 with 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 ± 63.99 mg; 55-μm mesh: 75.96 ± 82.97 mg) than root access mesocosms (1.45 mm mesh: 134.37 ± 120.58 mg; $P = 0.05$, $F = 3.31$). Mycorrhizal exclusion mesocosms reduced mycorrhizal biomass by approximately 50% (5-μm mesh: 2.38 ± 5.78 mg) compared with mesocosms containing mycorrhizas but excluding roots (55-μm mesh: 4.93 ± 10.24 mg) and by 60% compared to mesocosms containing mycorrhizas and roots (1.45 mm mesh: 6.46 ± 11.92 mg). GWC 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 GLMs to explore how root biomass, mycorrhizal biomass and their interaction altered soil carbon processes.

We successfully traced ^{13}C in CO_2 4 days after injecting the labelled starch into mesocosm soil that received the labelled 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 mycorrhizas 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 myc-

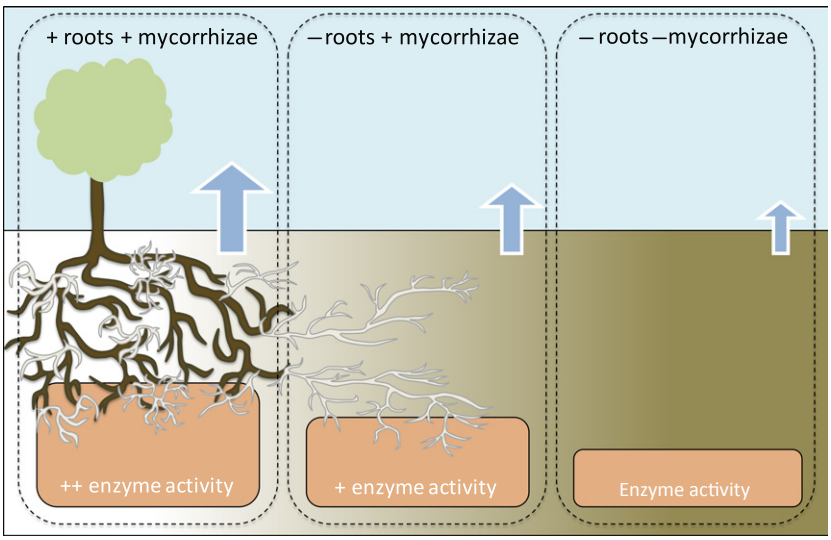

Fig. 1. Conceptual diagram illustrating hypothetical outcomes of root–mycorrhizal–microbial interactions. Our experimental mesocosms target three distinct and naturally occurring types of soil communities: communities with roots (dark coloured) and mycorrhizal fungi (light coloured) (+roots +myc), without 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 when 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: when decomposition was expected to be high, we would expect SOC pools to be low.

orrhizal 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 when 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 when mycorrhizal biomass was at a mid-level, and it was lowest when 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), 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 analysed and an unknown species of the family Sebacinaceae in 20% of the samples analysed. *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 in whether root and mycorrhizal abundance would influence soil carbon dynamics over a longer-term (30 years) 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 modelling approach 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 10 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 mycorrhizas but no roots and by 26% for the model without roots or mycorrhizas to calculate POC. Based on the peroxidase

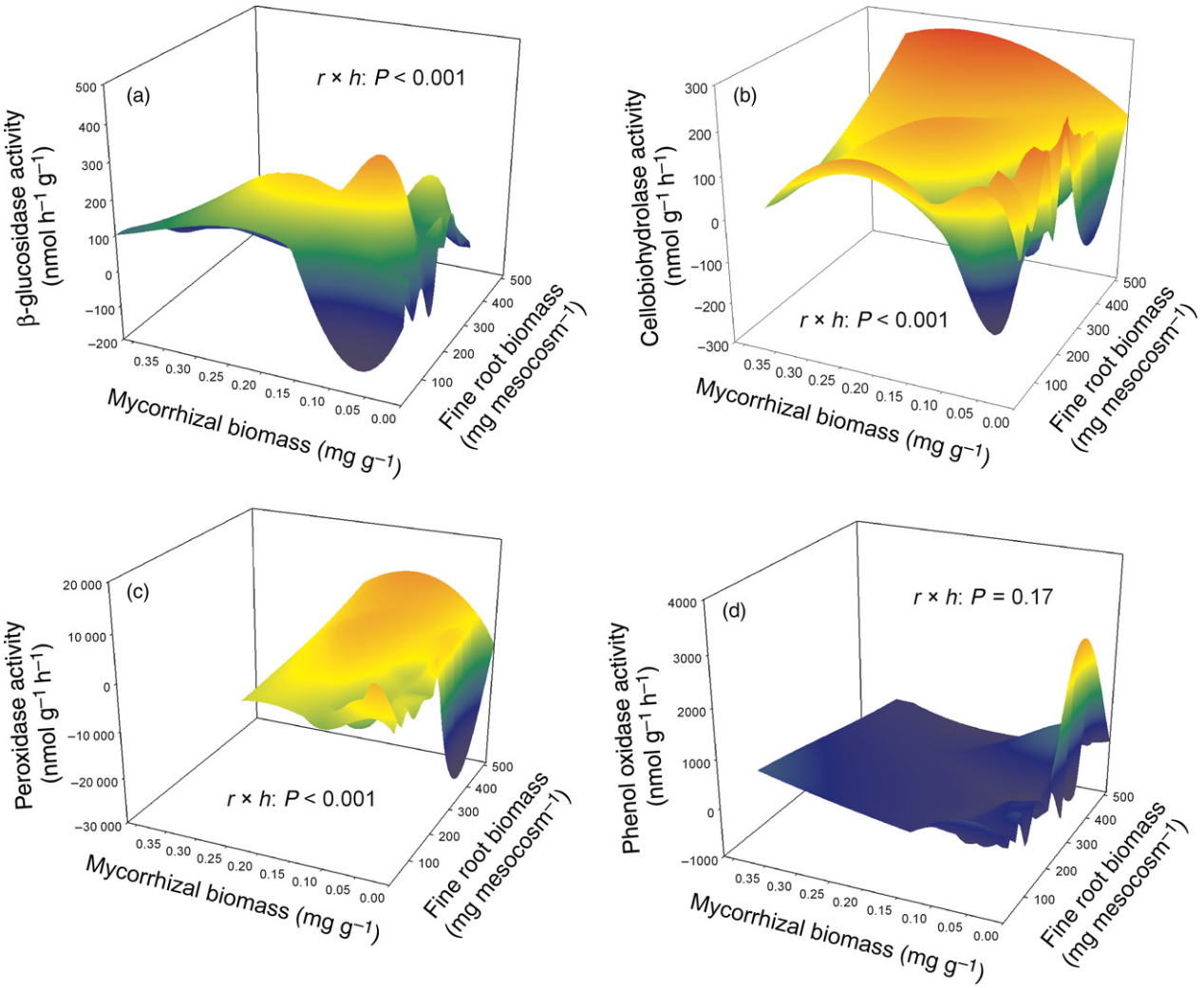

Fig. 2. The interaction between root (r) and mycorrhizal hyphal (h) biomass had various effects on soil carbon processes and pools. (a) Potential β-glucosidase activity (nmol g^{-1} h^{-1}) was negatively correlated with the interaction of roots and mycorrhizal biomass ($\chi^2 = 203.31$, $P < 0.001$). (b) Potential cellobiohydrolase activity (nmol g^{-1} h^{-1}) was positively correlated with the interaction between roots and mycorrhizal biomass ($\chi^2 = 43.30$, $P < 0.001$). (c) Potential peroxidase activity (nmol g^{-1} 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 (nmol g^{-1} h^{-1}) had no correlation with the interaction between roots and mycorrhizal biomass ($\chi^2 = 233.99$, $P < 0.001$).

values, we reduced V_{max} by 31% for the model with mycorrhizas but no roots and by 36% for the model without roots or mycorrhizas. When enzyme activities and V_{max} were adjusted to levels observed where 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 root biomass was low compared with 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 with 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 years).

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-labelled starch was highest when root biomass was high and mycorrhizal biomass was low, suggesting that interactions among roots, mycorrhizas and soil microbial communities

Table 1. Fine root (r) and mycorrhizal hyphal (h) biomass effects on activity of carbon-degrading enzymes, microbial biomass carbon (MBC), particulate organic carbon (POC), mineral organic carbon (MOC) and dissolved organic carbon (DOC) using generalized linear models

Response	Model	χ^2	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

Bold models are statistically significant ($P < 0.05$; $n = 30$).

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 a 30-year time frame: 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 of 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 when 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 & 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 probably 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 mycorrhizas 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 mechanisms: (i) that mycorrhizal fungi promoted the microbial decomposition of compounds in order to increase their nitrogen acquisition (Lindahl *et al.* 1999), (ii) that mycorrhizal fungi were active in lignin decomposition (Talbot, Allison & Tresder 2008; Wolfe, Tulloss & Pringle 2012) and (iii) that mycorrhizal fungi were suppressing microbial growth (de Boer *et al.* 2005). These three possible mechanisms are not mutually exclusive; for example, mycorrhizas 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 mycorrhizal–microbial interactions (Churchland & Grayston 2014). However, our data suggested that communities of mycorrhizas were similar across our treatments, consisting primarily of ectomycorrhizal

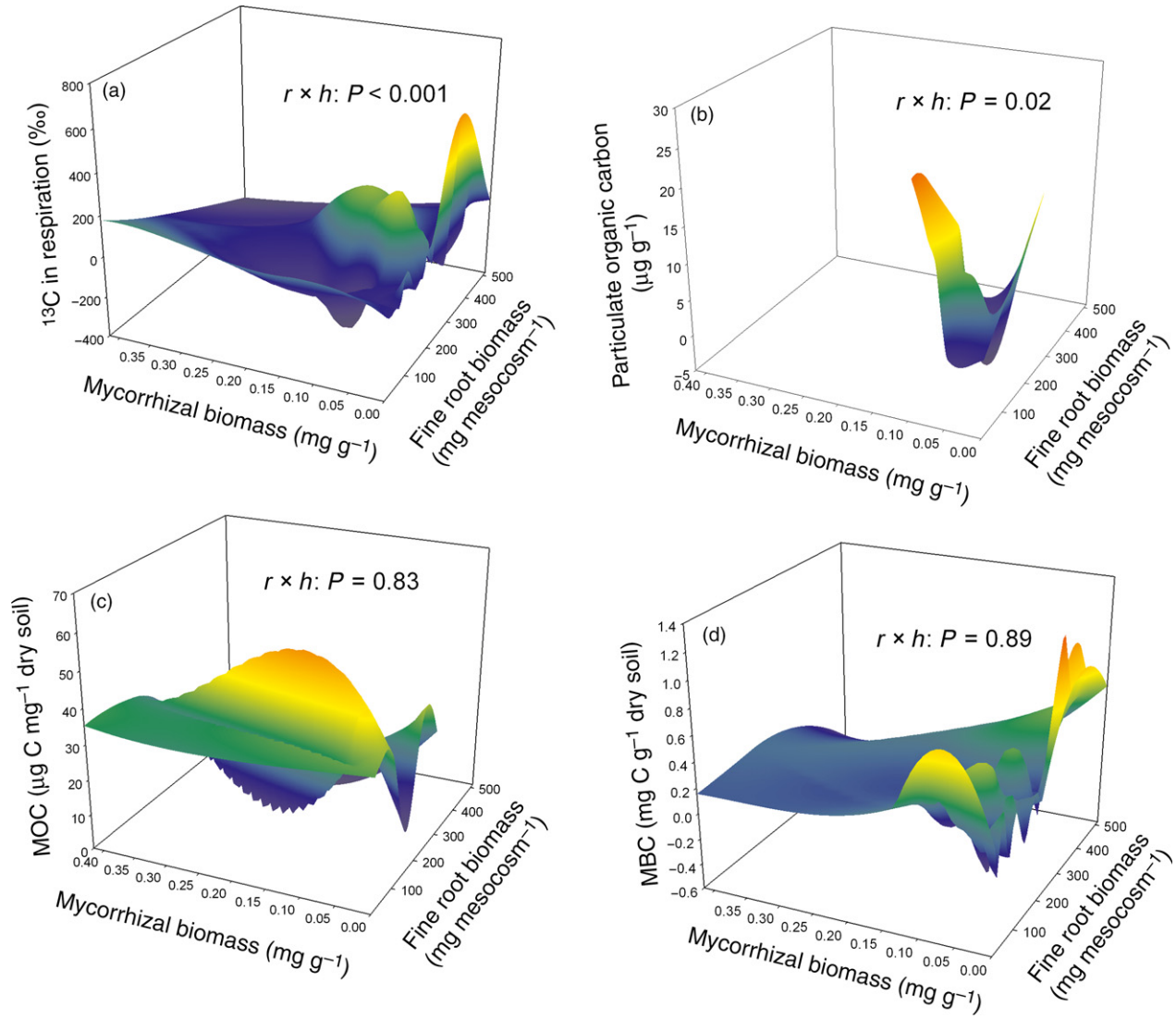

Fig. 3. Flux and pools of soil C are affected by the interaction between root and mycorrhizal biomass. (a) Respiration of ^{13}C starch was positively correlated with root and mycorrhizal biomass ($\chi^2 = 196.44$, $P < 0.001$). (b) Particulate soil organic carbon (POC) was negatively correlated with 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; $\chi^2 = 0.02$, $P = 0.89$).

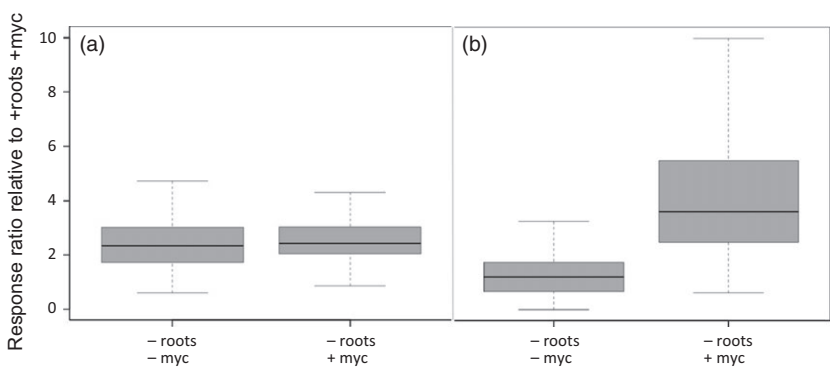

Fig. 4. Effects of roots and mycorrhizal exclusion on simulated pools of mineral (a) and particulate (b) organic carbon (MOC and POC, respectively) at steady state. Boxplots show data from permutated simulations. The black line indicates the median value, the box indicates the upper and lower quartiles, and the whiskers indicate the range of data. The response ratio was calculated as the ratio of MOC/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).

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 the 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 with the 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 while having a negligible effect on MOC.

The variability in simulations in the model with mycorrhizas 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 probably 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 (de Boer *et al.* 2005; Langley, Chapman & Hungate 2006) 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 have underestimated the degree to which mycorrhizal fungi contribute to soil carbon storage for two reasons: (i) turnover of recalcitrant mycorrhizal tissues could result in a slower decomposition rate (Langley, Chapman & Hungate 2006), and (ii) mycorrhizas 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–mycorrhizal–microbial 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 such as 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).

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Data accessibility

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

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