Plant roots stimulate the decomposition of complex, but not simple, soil carbon

Jessica A. M. Moore*[a], Benjamin N. Sulman[b], Melanie A. Mayes[b], Courtney M. Patterson[a], and Aimée T. Classen[c]

[a] Department of Ecology & Evolutionary Biology, University of Tennessee, Knoxville, USA

[b] Environmental Sciences Division and Climate Change Science Institute, Oak Ridge National Laboratory, Oak Ridge, TN, 37830, USA

[c] The Rubenstein School of Environment & Natural Resources, University of Vermont, Burlington, VT, 05405, USA

*Corresponding author: Jbryan44@utk.edu

Section: Ecosystems Ecology
Editor: Dr Emma Sayer

Abstract

1. Roots release carbon into soil and can alleviate energy limitation of microbial organic matter decomposition. We know little about the effects of roots on microbial decomposition of different organic matter substrates, despite the importance for soil carbon stocks and turnover. Through implementing root-microbe interactions, the Carbon, Organisms, Rhizosphere, and Protection in the Soil Environment (CORPSE) model was previously shown to represent dynamics of total soil carbon in temperate forest field experiments. However, the model permits alternative hypotheses concerning microbial-substrate affinity.
2. We investigated how root inputs affect decomposition of soil organic carbon (SOC) with variable decomposability. We simulated SOC stocks in CORPSE and compared microbial degradation of two substrates types with varying root-microbe interactions under two alternative hypotheses that varied in microbial-substrate affinity. We compared our modeled hypotheses to a forest field experiment where we quantified decomposition of isotopically-labeled starch and leaf tissues in soils with manipulated root access to microbes. We tested the hypothesis that decomposition of leaves would be more sensitive to root inputs than decomposition of starch, corresponding to the alternative model hypothesis.
3. In the field study, leaf decomposition increased with root density while starch decomposition was unchanged by root density. Microbial biomass and enzyme activity consistently increased with root inputs in CORPSE and the field study. Our field experiment supported the CORPSE simulations with high microbial-substrate affinity.
4. Roots stimulated microbial growth and enzyme production, which increased degradation of more complex substrates such as leaf tissues. Substrates that were easily decomposed, such as starch, may already be degrading at a maximum rate in the absence of rhizosphere influence because their decomposition rate was unchanged by root inputs. We found that the degree to which roots stimulate microbial

decomposition depends on the substrate being decomposed, and that root-microbe interactions influenced SOC stocks in both our model and field experiment. Environmental changes that alter root-microbe interactions could, therefore, alter soil C stocks and biogeochemical cycling, and models of these interactions should incorporate differential influence of rhizosphere inputs on different substrates.

Keywords: broadleaf boreal forest; ecosystem model; extracellular enzymes; plant-microbe interaction; soil carbon; soil organic matter; stable isotopes

Introduction

Plants fix carbon (C) from the atmosphere to build biomass and much of that biomass enters soil as leaf and root products. Although ample research has focused on aboveground inputs (Xu, Liu, & Sayer, 2013), roots contribute 2.5-fold more C to soil than shoots (Rasse, Rumpel, & Dignac., 2005). Given that soil is the largest stock of terrestrial C except for fossil reserves (Post, Emanuel, Zinke, & Stangenberger, 1982; Jobbagy & Jackson, 2000), exploring how root inputs affect soil C accumulation and feedbacks to the atmosphere is critical to understanding and modeling the global C cycle (Phillips et al., 2012). Root inputs to soil in broadleaf boreal forests are particularly important because roots comprise 39% of plant biomass, a greater portion than in needle-leaf boreal, temperate, or tropical forests (Vogt et al., 1995). While researchers recognize that roots are underrepresented in C models (Lynch, Matamala, Iversen, Norby, & Gonzalez-Meler, 2013; McCormack et al., 2015), we are only beginning to understand and model how root inputs alter soil C stocks (Keiluweit et al., 2015).

Root inputs from sloughed-off root cells, mucilage, exuded organic compounds, and dead root tissues affect rates of soil C decomposition and accumulation. Microbial enzyme activity increases with root exudation (Phillips, Finzi, & Bernhardt, 2011; Meier, Finzi, & Phillips, 2017). Root exudates prime microbial activity, where microbes release more C in CO_2 than is contained in the exudates (Kuzyakov, 2010), in at least two ways: by increasing available dissolved organic C and co-metabolism, and by lowering soil pH such that mineral-associated organic matter is liberated from mineral surfaces (Blagodatskaya & Kuzyakov, 2008; Kuzyakov et al., 2010; Keiluweit et al., 2015). As root exudates increase DOC, microbes are alleviated from energy limitation and increase decomposition activity (Kuzyakov et al., 2010). Thus, both exudate-driven mechanisms for decomposition translate to increased mineralization of soil C. In fact, Crow et al. (2009) found that 11.5% – 21.5% of soil respiration in a temperate hardwood forest was attributed to stimulation of microbial activity due to root inputs. While experiments indicate that roots influence microbial activity (Lindahl, de Boer, & Finlay, 2010; Clemmensen et al., 2013; Drake et al., 2013), incorporation of roots into soil C decomposition theory and models has lagged.

Historically, models of soil organic matter decomposition have largely been based on C pools with fixed turnover rates that do not accommodate the microbial decomposition feedbacks necessary to simulate root input-microbial interaction influences on decomposition. While

microbial interactions have been incorporated into emerging soil C models, alternative structural assumptions in these models lead to diverging responses to C inputs (Sulman et al., 2019). Models of rhizosphere input effects are particularly sensitive to assumptions related to substrate concentrations, microbial growth, and organic matter decomposition. Decomposition in these models has been described using Michaelis-Menten enzyme kinetic theory where decomposition rates increase with enzyme concentrations (e. g. Wang et al., 2015) or substrate concentrations (e.g. Wieder et al., 2014) or in a more general framework using equilibrium chemistry approximation (ECA) kinetics that incorporate both enzyme and substrate concentrations (Tang and Riley, 2015; Tang 2015). An alternative approach incorporated in the CORPSE model (Carbon, Organisms, Rhizosphere, and Protection in the Soil Environment; Sulman, Phillips, Oishi, Shevliakova, & Pacala, 2014) assumes that microbial decomposition of soil organic matter is determined by the amount of microbial biomass per unit substrate, rather than volumetric concentration of substrate or enzymes. This approach allows the model to represent multiple substrate types with different decomposition-related properties and is therefore useful for simulating rhizosphere input effects.

An issue common to all of these model formulations is whether the effect of rhizosphere inputs on microbial decomposition is substrate-specific (Fig. 1). In one formulation (Hypothesis 1), the decomposition rate of all compounds is controlled by the total concentration of microbial biomass, meaning that all compounds have identical decomposition responses to microbial growth. Alternatively (Hypothesis 2), rhizosphere input effects of different compounds may saturate at different levels of microbial biomass, with simple compounds achieving their maximum decomposition rate at low microbial biomass concentrations and decomposition of more complex compounds increasing more slowly with respect to microbial biomass. These alternative outcomes have important implications for the preservation or decomposition of labile substrates in resource-limited environments such as deep soils or in highly-decomposed material with low labile substrate concentrations. We used the CORPSE model in the context of a field decomposition experiment to test which of these alternative hypotheses is a more appropriate representation of microbial decomposition processes. We hypothesized that (1) the decomposition rate of each substrate type is determined by the ratio of microbial biomass to total unprotected soil C. In this case, changes in microbial biomass affect decomposition rate of all substrates identically. Thus, simple and complex substrates would decompose slowly when

microbial biomass is small relative to total unprotected C, and decomposition rates of all substrates would accelerate at the same proportional rate as microbial biomass increases. This scenario represents a situation in which microbial decomposers assimilate a well-mixed combination of substrates. Alternatively, we hypothesized that (2) the decomposition rate of each substrate is related to microbial biomass to different degrees for different substrates. That is, simple C could be decomposed rapidly given low microbial biomass while complex C could be less sensitive to changes in microbial biomass. This hypothesis represents a scenario in which substrates are distributed unevenly and can be accessed separately by decomposers. Microbes can target substrates that are present in small amounts but have properties that are highly favorable for assimilation. The overall implication of our model hypothesis (i) is that the effect of rhizosphere input on microbial decomposition are universal for all substrates, and the implication of model hypothesis (ii) is the effect of rhizosphere inputs can vary for different substrates.

We empirically investigated how soil C decomposition responded to root inputs in a broadleaf boreal forest. Root exudation is known to vary with root density (Phillips et al., 2011), thus we used root density as a proxy for both exudation and root litter inputs. We simulated soil C processes using the CORPSE model to determine differential responses of simulated SOC across a gradient of root inputs. CORPSE divides SOC into different types, including one that is easily decomposed and assimilated (i.e., simple) by microbes and a second that is less easy to decompose (i.e., complex). We compared the CORPSE-simulated C pool responses to measurements in a field study where we experimentally generated a gradient of root density and tracked decomposition of leaf material and starch. Using this combined model-experiment approach, we answered the question: how does microbial activity and decomposition of soil C that is chemically simple or complex respond to a gradient of root density? We hypothesized that: (i) C mineralization rates of leaf material would be lower than those of starch, (ii) microbial biomass would increase with root density, (iii) microbial enzymatic activity would increase with root density, and (iv) C mineralization rates from leaf material would increase with higher root density but C mineralization rates from starch would be constant with root density. After six weeks of field incubation, we measured $^{13}CO_2$ respired from soils amended with ^{13}C-labeled leaf material or starch, microbial biomass, and enzymatic decomposition activity. Our model

simulations and experiment suggested that root density influenced decomposition of chemically complex C more than simple C.

Methods

CORPSE simulations

The CORPSE model simulates soil C cycling using an explicitly defined microbial biomass pool that drives the decomposition rate of multiple organic substrates (Fig. 2). See Supplemental Table S2 for model parameter values used in our simulations. Organic matter is divided into three chemically-defined forms, which can be either protected or unprotected. Protected organic matter is inaccessible to microbial decomposition through chemical sorption to mineral surfaces or occlusion within micro-aggregates. Unprotected organic matter can be added as litter or root exudate inputs, decomposed by microbial action, or protected:

$$\frac{dC_{U,i}}{dt} = I_{C,i} - D_i + T_M - \frac{dC_{P,i}}{dt} \quad \text{(eqn. 1)}$$

where $C_{U,i}$ is unprotected C; $I_{C,i}$ is external inputs of C (including litter deposition and root exudation); D_i is decomposition rate; T_M is microbial necromass production; and $\frac{dC_{P,i}}{dt}$ is net transfer of C to or from the protected state. i refers to chemically-defined types, which can be chemically simple plant-derived material (representing compounds like glucose or amino acids that are readily decomposed), chemically resistant (representing compounds like lignin or complex microbially-produced chemicals), or readily decomposable microbial necromass. Protected C is formed from unprotected organic matter and converted back to unprotected form at first-order rates:

$$\frac{dC_{P,i}}{dt} = C_{U,i} \cdot k_{P,i} - \frac{C_{P,i}}{\tau_P} \quad \text{(eqn. 2)}$$

Note that this model formulation does not currently include rhizosphere effects on the turnover of protected C. The decomposition flux is controlled by microbial biomass (B_M), temperature (T), and volumetric soil water content (θ). The effect of microbial biomass on decomposition was defined in two alternate ways, reflecting Hypothesis 1 (equation 3a), and Hypothesis 2 (equation 3b).

$$D_i = V_{max,i}(T) \cdot \left(\frac{\theta}{\theta_{sat}}\right)^3 \left(1 - \frac{\theta}{\theta_{sat}}\right)^{2.5} \cdot C_i \frac{B_M / \sum_i C_{U,i}}{B_M / \sum_i C_{U,i} + k_C} \quad \text{(eqn. 3a)}$$

$$D_i = V_{max,i}(T) \cdot \left(\frac{\theta}{\theta_{sat}}\right)^3 \left(1 - \frac{\theta}{\theta_{sat}}\right)^{2.5} \cdot C_i \frac{B_M / C_{U,i}}{B_M / C_{U,i} + k_C} \quad \text{(eqn. 3b)}$$

where θ_{sat} is the saturation level of θ and $V_{max,i}$ is the substrate-specific maximum decomposition rate. Increases in B_M driven by growth on substrates with high carbon use efficiency and V_{max} drive priming effects in the model (see below). Note the key difference between equations 3a and 3b: In equation 3a, decomposition rate is determined by the ratio of B_M to C_U summed over all substrate types, while in equation 3b decomposition rate is determined for each substrate type $C_{U,i}$ by the ratio of B_M to the amount of that substrate type.

The maximum decomposition rate is controlled by the Arrhenius relationship, which describes the temperature dependence of enzymatic reactions:

$$V_{max,i}(T) = V_{max,ref,i} \times exp\left(-\frac{E_{a,i}}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right)\right) \quad \text{(eqn. 4)}$$

where $V_{max,ref,i}$ is a maximum decomposition rate specific to each chemically-defined organic matter type, $E_{a,i}$ is activation energy for each organic matter type, and R is the ideal gas constant (8.31 J K^{-1} mol^{-1}).

Microbial growth is supported by uptake of a fraction of decomposed organic matter, and biomass is lost through turnover at a fixed rate:

$$\frac{dB_M}{dt} = \sum_i (D_i CUE_i) - \frac{B_M - B_{min}}{\tau_{mic}} \quad \text{(eqn. 5)}$$

where CUE_i is C use efficiency for substrate i and τ_{mic} is the microbial biomass turnover time. The complex C is defined in CORPSE as having low maximum decomposition rate (V_{max}) and low microbial C use efficiency (CUE) which is comparable to leaf material, whereas simple C has high V_{max} and high CUE which is comparable to starch. We defined starch as a simple

substrate because it is a pure carbohydrate chain, and leaf material as complex because it contains many compounds bound in a lignocellulose matrix. Because simple C has a higher associated CUE and V_{max} than complex C, it promotes microbial growth, thereby accelerating decomposition and driving priming effects for all substrates. B_{min} is minimum microbial biomass, defined as a fraction of total unprotected C:

$$B_{min} = f_{B,min} \sum_i C_{U,i} \quad \text{(eqn. 6)}$$

Microbial biomass turnover is divided into maintenance respiration (R_{maint}), which is converted directly to CO_2, and necromass production (T_M). The division between R_{maint} and T_M is controlled by a parameter ϵ_t:

$$R_{maint} = \frac{B_M - B_{min}}{\tau_{mic}}(1 - \epsilon_t) \quad \text{(eqn. 7)}$$

$$T_M = \frac{B_M - B_{min}}{\tau_{mic}}(\epsilon_t) \quad \text{(eqn. 8)}$$

Total CO_2 production rate is the sum of maintenance respiration and respiration derived from decomposition processes:

$$\frac{dCO_2}{dt} = R_{maint} + \sum_i((1 - CUE_i)D_i) \quad \text{(eqn. 9)}$$

Rhizosphere input simulations

We parameterized the model using soil texture measured at our experimental field site (described below, in Field Study) and measured soil temperature and moisture from a nearby monitoring station (Hanson et al., 2011). Total C inputs to the soil were estimated to be 1.5 mg C g soil^{-1} y^{-1}, composed of 30% simple C and 70% complex C, and the model was spun-up with repeating inputs and meteorological drivers until soil C pools reached a steady state. We then simulated decomposition across a gradient of root density that was representative of the measured variability of root density at our site. Root exudation was calculated based on root length using an estimated growing-season value of 0.25 μg C (cm root length)$^{-1}$ hour^{-1} (Phillips

et al., 2011; Yin et al., 2013). Root exudation was assumed to have a sinusoid pattern through the year, with maximum exudation rate occurring in August of each year (Phillips et al., 2011). Root exudates were assumed to be entirely composed of simple C. This assumption is a simplified representation of exudate composition, which may also include organic acids that can liberate mineral-bound soil C thus further alleviating microbial C limitation (Keiluweit et al., 2015). Our simplified representation of exudates therefore yields more conservative results because it limits the source of microbial priming to simple C compounds (e.g., glucose).

Field Study

The field study was conducted at Marcell Experimental Forest (47°30'26.73", -93°27'15.68") located 40 km north of Grand Rapids, Minnesota, USA. The average annual temperature was 3°C and average precipitation was 785 mm yr^{-1}. Our 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). Soils were pH 5.0 ± 0.44 with a bulk density of 1.26 ± 0.41 g cm^{-3} and are fine, sandy loams classified as Warba series (Kolka, Grigal, Nater, & Verry, 2001).

We manipulated root access to soil microbes by constructing mesocosms 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 (Household Goop, Eclectic Products, Eugene, USA). We covered 135 mesocosms with one of three mesh sizes: 1.45 mm (n = 45), 38 µm (n = 45), or 5 µm (n = 45). We intended to exclude roots from fine-mesh mesocosms and allow root access to soil with large-mesh mesocosms (Johnson, Leake, & Read, 2001; Langley, Chapman, & Hungate, 2006; Phillips et al., 2012; Rewcastle et al. In press). However, a previous study showed the mesh design does not generate absolute root exclusion in all forest types (Moore et al., 2015). Mesocosms in the previous study varied in root density, thus we analyzed our data across a gradient of root density.

On May 12, 2014, we installed mesocosms randomly throughout the study site. We removed any organic horizon material and excavated the top 15 cm of mineral soil using a 5 × 15 cm hammer corer (AMS, Inc., American Falls, USA). The top 15 cm included only the A

horizon. We removed visible roots to avoid a litter fertilization effect, and then filled each mesocosm with this root-free native soil. We placed each mesocosm in the hole from which it was collected and ensured the mesh was completely below the soil surface. Mesocosms were placed at least 0.5 m away from each other.

In-situ ^{13}C-starch and ^{13}C-leaf material incubation

Adding stable isotopes to soil enabled us to track microbial activity within specific C pools. We applied a 99 atom-% ^{13}C labeled algal starch (Cambridge Isotope Laboratories, Tewksbury, MA, USA) and a >97 atom-% ^{13}C labeled ground tulip-poplar (Liriodendron tulipiferae) leaf material (IsoLife, Wageningen, Netherlands). We suspended 5 mg of powdered starch (0.58 mg C) or ground leaf material (2.3 mg C) in 30 mL of deionized water and injected into mesocosms that contained approximately 350 g soil. The amount of C added to soil from starch (1.6 µg C g^{-1}) or leaf material (6.6 µg C g^{-1}) was large enough to have a traceable label but small enough to not fertilize the soil, which contained on average 20 mg C g^{-1} soil at our site and is a similar amount to other C tracer field studies (Zak & Kling, 2006). The injections were conducted on June 24, 2014, six weeks after installing the mesocosms. We injected the starch suspension into 45 mesocosms (15 of each mesh size) and the leaf solution into 45 mesocosms (15 of each mesh size). To control for moisture addition and disturbance, we injected deionized water into 21 starch-control mesocosms (7 of each mesh size) collected on the same day as starch-addition mesocosms, and into 24 leaf-control mesocosms (8 of each mesh size) collected on the same day as leaf-addition mesocosms. Mesocosms injected with the starch suspension were sampled for $^{13}CO_2$ on days 1, 2, 3, 4 and 5 after injection, and those with the leaf suspension were sampled on days 2, 4, 6, 10, and 20 after injection. We sampled gasses across several days because we were unsure which day CO_2 flux would peak and this timeframe ensured we would capture peak microbial respiration of the ^{13}C-labeled substrate (Zak & Kling, 2006). To collect gas samples for $^{13}CO_2$ analysis, we capped the cores with a tightly fitting 5 cm diameter PVC cap fitted with a rubber septum. After 20 min, 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, UK). One gas sample per sampling day was taken from the cores. At the beginning and end of each sampling day two ambient samples were taken to establish background levels of $^{13}CO_2$. All $^{13}CO_2$ samples were analyzed at the UC Davis Stable Isotope Facility (Davis, USA) using a ThermoScientific PreCon-GasBench system interfaced to a

ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, USA). We removed all starch and starch-control mesocosms on June 29, 2014 and all leaf and leaf-control mesocosms on August 4, 2014. We placed the contents of mesocosms in a plastic bag, transported them in a cooler on ice, and stored at 4°C until they were analyzed.

To quantify root density, we removed unsieved soil from the mesocosms and visually inspected soils for roots. We used forceps to collect fine (<2 mm) roots and placed field-moist root mass into a clear-bottomed reservoir filled with water to a depth of approximately 2 cm. We scanned the roots in the reservoir on a photo scanner at 300 dpi resolution. We cropped the images to remove the border created by the reservoir, and then calculated root length using the Morphology plug-in and IJ Rhizo script for ImageJ software (Lobet & Draye, 2013). Root density is equal to root length per volume soil.

We analyzed microbial biomass C (MBC) within 48 hours of soil collection using the chloroform fumigation-extraction method (Vance, Brooks, & Jenkinson, 1987), allowing fumigated samples to incubate at room temperature for 5 days. All samples were stored at 4°C until analysis. We measured C of the samples on a total organic carbon analyzer (TOC-V CPH Total Organic Carbon Analyzer, Shimadzu Scientific Instruments, Columbia, USA). Microbial biomass C was calculated using a correction factor of 0.38 (Voroney, Brooks, & Beyaert, 2007).

We analyzed the potential enzyme activity of our soils using methods described by Bell et al. (2013) within 48 h of collection. Briefly, we mixed 2.75 g of field moist soil (sieved to 2 mm) with 91 mL of 50 mM sodium acetate buffer at pH 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 0 -100 µM of methylumbelliferyl (MUB) to establish a standardized MUB reaction for each soil sample. We then pipetted 800 µL of the soil slurry into a separate plate and added 200 µL of 4-MUB-ß-D-glucoside (ß-gluc), 4-MUB-cellobioside (CBH), 4-MUB-N-acetyl-ß-D-glucosaminide (NAG), or 4-MUB-phosphate (PHOS) to each soil sample. ß-gluc and CBH are hydrolytic enzymes that work in concert to break down cellulose into glucose, and NAG and PHOS are used by microbes to acquire nitrogen and phosphorus, respectively. We sealed each plate with a plate mat, agitated vigorously by hand, then incubated the MUB standard and sample plates in the dark at room temperature for 3 h. Using a fluorometer/spectrophotometer (Synergy HT, Biotek Inc, Winooski, USA) we measured fluorescence at an excitation wavelength of 365 nm and an emission wavelength of 450 nm.

313 Statistical Analyses

314 We tested for the effects of root density on microbial activity using linear regressions.
315 Data were log-transformed when necessary to meet assumptions of normality. We tested whether
316 roots affected microbial metabolism of different pools of C by regressing root density against
317 $\partial^{13}C$ captured in CO_2 and included C source (starch or leaf material) as a co-variate. We
318 determined the effect of roots on microbial biomass by regressing root density with MBC, and
319 the effect of roots on microbial activity by regressing root density with each of four enzyme
320 activities. All regressions were performed separately and were considered significant at $\alpha = 0.05$.
321 We report the probability that empirical responses were not related to root density (P), ratio of
322 variance among empirical response groups (F), and coefficients of correlation between empirical
323 responses and root density (r^2). All analyses were performed in R (R Core Team, 2016) using the
324 basic package and normality was tested for using the package fBasics (Rmetrics Core Team,
325 2014).

326 **Results**

327 CORPSE Simulations

328 Model simulations showed a strong effect of root density on microbial biomass and
329 decomposition rates, and projected significant differences in simple C decomposition between
330 the alternative hypotheses. Root exudation in the simulations with the highest root density
331 increased the decomposition rate of complex C by more than 120% under both hypotheses (Fig.
332 3a). In contrast, the decomposition rate per unit mass of simple C declined slightly (by less than
333 1%) as root density increased under Hypothesis 2 while increasing similarly to complex C under
334 Hypothesis 1. The decline in simple C turnover rate with higher root density under Hypothesis 2
335 occurred because the amount of total simple C increased with additional root inputs, whereas the
336 amount of total complex C was unchanged by root inputs. The accelerated decomposition rate of
337 complex C was driven by a large increase in simulated microbial biomass concentration at higher
338 root densities (Fig. 3b, S1). Simulated microbial biomass across the gradient of root density was
339 consistent with measurements (see below).

340 Field Study

341 Roots affected decomposition of leaf-C differently than starch-C. Root density in field
342 mesocosms ranged from 0.1 to 523.3 mm g^{-1} dry soil (mean = 63.4 mm g^{-1}, median = 14.8 mm g^{-1})
343 and it did not vary with mesocosm mesh size (P = 0.15, F = 1.93), soil C:N (P = 0.67, F =

0.18), or soil pH (P = 0.60, F = 0.27; Supplemental Table S1). The effect of root density on $\partial^{13}CO_2$ was different for starch and leaf material (P = 0.009, F = 7.24). The $\partial^{13}CO_2$ captured from decomposed labeled leaf material increased with root density, while decomposition of labeled starch was not correlated with root density (P = 0.009, R^2 = 0.51, Fig. 3c). When we standardized the $\partial^{13}CO_2$ respired given the different initial C concentrations of leaf material and starch, we found that $\partial^{13}CO_2$ respired from the substrates was related to root density differently (P = 0.01, F = 6.72). For both substrates, decomposition rates peaked two days after the substrates were added to soils.

Root density increased MBC and C-degrading enzyme activity. Microbial biomass C increased with root density (P = 0.001, R^2 = 0.12, Fig. 3d). As we anticipated, the effect of root density on MBC did not vary with C source because of the trace amount of substrate C added to each mesocosm (P = 0.61, F = 0.49). ß-glucosidase potential activity per unit soil C increased with root density (P = 0.01, R^2 = 0.17, Fig. 4a), but was not affected by C source (P = 0.23, F = 1.48). Cellobiohydrolase potential activity was not related to root density (P = 0.10, R^2 = 0.04, Fig. 4b), and did not vary with C substrate (P = 0.64, F = 0.45). Root density was not correlated with the nutrient-acquiring enzymes NAG (P = 0.13, R^2 = 0.01) or PHOS (P = 0.08, R^2 = 0.02). PHOS rates were marginally higher in leaf-addition mesocosms and lower in starch-addition mesocosms (P = 0.03, F = 3.47), but a post-hoc Tukey HSD test suggested that neither were different from control (P = 0.26 for leaf material v. control, P = 0.59 for starch v. control). PHOS rates were also similar to control with starch-addition (Tukey HSD: P = 0.88) and were higher than control for leaf-addition (Tukey HSD: P = 0.03). Overall, we found that roots stimulated microbial biomass and C-degrading activity but not nutrient-acquiring activity.

Discussion

Root inputs stimulate microbial decomposition (Phillips et al., 2011; Keiluweit et al., 2015), but modeling approaches and previous empirical studies have not definitively established to what extent root-microbe interactions differently influence the decomposition of different SOC fractions. We addressed this uncertainty by comparing simulations from the rhizosphere model CORPSE with an experiment conducted in a broadleaf boreal forest because boreal forests harbor large pools of C that is potentially climate-sensitive (Clemmensen et al., 2013; Bradshaw & Warkentin 2015; Crowther et al., 2016). We demonstrated that root inputs were correlated

with decomposition of complex SOC, while there was negligible correlation between root density and decomposition of simple SOC. Thus, our experimental results supported Hypothesis 2, indicating that substrates decompose at different rates depending on root-microbe interactions. Decomposition of complex SOC in CORPSE under both hypotheses increased as root inputs increased microbial activity and biomass, a result that was supported by our field experiment. We measured higher efflux of $^{13}CO_2$ originating from leaf material where root density was high compared to low and found no relationship between $^{13}CO_2$ efflux from starch and root density. Overall, both our empirical and model results demonstrated that complex C was more sensitive to root-microbe interactions than simple C and suggested that model formulations consistent with these differential effects on different substrates should be used in simulations of rhizosphere impacts on soil C.

Previous studies find that rhizosphere interactions are important in the context of deep soils (Hicks Pries et al., 2018), Arctic soils (Hartley et al., 2012), and ecosystem-scale C and nutrient cycling (Finzi et al., 2015), and that rhizosphere interactions can drive global-scale sensitivity of soil C stocks to changes in climate and ecosystem productivity (Sulman et al., 2014; Sulman et al., 2019). Improving model representations of rhizosphere interactions is important for enhancing the predictive capacity of ecosystem models. In particular, our results suggest that in substrate-limited environments like deep soils, preservation of labile C substrates may be limited since external resource subsidies are not required for their decomposition. Thus, even in deep soils the preservation of organic material due to resource limitation may be limited to more complex substrates.

Complex C decomposition increased with root inputs in the CORPSE simulations. To directly compare with this modeled result, we would need to measure turnover of complex SOC in isolation from other SOC pools, but measuring turnover of distinct pools of SOC is a challenge in field studies. We did, however, measure mineralization of two types of C using isotopically labeled substrates. We found increased $^{13}CO_2$ from leaf material with increasing root density, which suggested increased microbial activity and turnover of complex C. Our result corroborates a temperate forest tree girdling study. When root inputs to soil were cut off, leaf litter decomposition was reduced by 40% compared to control plots (Brzostek, Dragoni, Brown, & Phillips, 2015). CORPSE simulations suggested that root exudate inputs alleviated C limitations to microbial biomass and thereby enhanced complex C decomposition by stimulating

406 the growth of microbial biomass. Root inputs were correlated with microbial biomass here and in
407 a temperate pine forest (Phillips et al., 2011), but neither of these studies isolated the responses
408 of different SOC fractions. We hypothesize that microbes specializing on leaf material
409 degradation increased decomposition activity in presence of leaf material, and these microbes
410 were sensitive to root inputs. While it is known that root-associated microbial communities are
411 distinct from those in bulk soil (DeAngelis et al., 2009; Shi et al., 2011; Lou et al., 2014),
412 whether these communities differentially decompose pools of C remains unknown.
413 In contrast to complex C, simple C decomposition did not respond to higher root density
414 in this boreal forest experiment. The CORPSE model simulations suggested that this response
415 was more consistent with the assumption that microbial access and assimilation of these simple
416 C compounds did not benefit from additional energy subsidies or other rhizosphere effects. In
417 other words, the rapid decomposition rate and high CUE supported enough microbial biomass to
418 decompose simple C at a maximal rate even at low concentrations of simple C. As a result, the
419 simulated relationship between microbial biomass and simple C decomposition reached a
420 saturation point at low levels of root inputs (Figure 1b; Supplemental Fig. S1). These results
421 suggest that in environments composed mostly of complex material such as needleleaf-
422 dominated litter layers, even small amounts of labile C could significantly stimulate microbial
423 decomposition of more complex substrates. Under Hypothesis 1, labile C decomposition was
424 slower when labile C concentration was low relative to complex C (in the absence of root
425 exudates). Our study suggested that this assumption was incorrect, and that instead labile C could
426 decompose rapidly, enhancing microbial growth, even at low concentrations (Hypothesis 2).
427 Nutrient limitation is a potential hypothesis that may resolve the different responses of
428 complex and simple C decomposition rates to root inputs in our study. Microbes may
429 preferentially utilize inputs that contain C and N, rather than C-rich inputs, to meet their
430 stoichiometric demands (Drake et al., 2013). We did not explore nutrient limitation of
431 decomposition in CORPSE, and we have not experimentally found a relationship between
432 microbial N or P acquisition and root density or decomposition of complex or simple C. Yet,
433 others have reported increased N-acquiring activity with root exudation (Phillips et al., 2011;
434 Meier et al., 2017) and higher rates of N immobilization in presence of roots (Holz et al., 2016).
435 In a temperate pine forest, proteolytic activity doubled with additions of root exudate-like
436 compounds to soil (Meier et al., 2017). We may not have captured any effect of root inputs on

nutrient-acquisition because we measured only two of the many nutrient-acquiring enzymes that microbes produce. Alternatively, the in-growth mesocosm installation likely disturbed soil aggregates, thus altering nutrient pools, and the disturbed microbial communities may have altered community structure and functional capacity for decomposition (Franzluebbers, 1999). We studied decomposition within in-growth mesocosms relative to disturbance-control mesocosms. Rhizosphere interactions in undisturbed soils may differ from those we have demonstrated within in-growth mesocosms due to the experimental manipulations we imposed. For a more comprehensive microbial activity investigation, we recommend that future studies take advantage of -omics technologies and gene expression assays targeting production of nutrient transport proteins (Treseder & Lennon, 2015).

While our study focused on the decomposition of SOC compounds with different chemical complexities, a large fraction of SOC is physically protected from microbial decomposition via associations with mineral particles or small aggregates. Discerning root-microbe interactive effects on decomposition of C that is protected via different mechanisms will be critical to the development of next generation root-microbe-mineral ecosystem models (Buchkowski, Bradford, Grandy, Schmitz, & Wieder, 2017). Protected C within CORPSE is broadly defined and includes C that is physically inaccessible to microbes, C that is stabilized on mineral surfaces. These contrast with C incorporated into chemically complex polymers that can have moderately long turnover times (particularly in the absence of simple C inputs) but are not physically protected from microbial access. In future studies, we recommend testing root-microbe influences on decomposition of C that is protected by different mechanisms to advance model development. While we demonstrated microbial use of recent C inputs, microbial use of C inputs that have been incorporated into different pools of C is another important next step to pursue. We suggest future investigations of microbial decomposition of isotopically labeled particulate and mineral-associated organic C or C that is protected from microbial decomposition via different mechanisms. For example, in an experiment by Haddix, Paul, and Cotrufo (2016) leaf litter with isotopically distinct structural and metabolic components was decomposed and C from metabolic components was traced into the mineral-associated C pool, while C from structural components was traced into the particulate C pool. Other investigations like this are needed to improve how microbial traits and processes are represented in C models.

Conclusions

Our model and experiment suggested that root-microbe interactions have different effects on decomposition of complex compared to simple soil C. Roots stimulated decomposition of leaf material and did not stimulate decomposition of starch in a broadleaf boreal ecosystem. It is likely that complex C decomposition increased with root density because of microbial growth, i.e., limitations of active microbial biomass limitation were alleviated. Simple C decomposition probably did not respond to root density or root exudation because microbes could grow efficiently on the simple substrate without requiring additional resources. These results provide an important constraint for representation of rhizosphere interactions in soil C models, suggesting that model structures should accommodate interactions among substrates, microbes, and roots to accurately represent soil decomposition mechanisms. We urge future investigators to isolate decomposition in SOC fractions using measurements that take advantage of microbial – omics technologies and advanced soil chemical analyses to increase our understanding of how root-microbe interactions influence turnover of different soil C pools. Clearly, roots influence microbial C processing, but the specific mechanisms of root-microbe interactions have yet to be fully explored and understood in the context of existing biogeochemical frameworks.

Acknowledgements

This work was funded by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Terrestrial Ecosystem Sciences Program under Award Number DE-SC0010562. A Graduate Research grant to JAMM from the Ecology & Evolutionary Biology Department at University of Tennessee contributed to this work. BS is supported under award NA14OAR4320106 from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce. The statements, findings, conclusions, and recommendations are those of the author(s) and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration, or the U.S. Department of Commerce. ORNL is managed by the University of Tennessee-Battelle, LLC, under contract DE-AC05-00OR22725 with the US Department of Energy. S. Patel, K. Rewcastle, and J. Henning assisted with sample collection and processing. G. Wang and R. J. Norby of ORNL provided comments on experimental design and the Soil Ecology group at University of New Hampshire commented on an initial manuscript draft. The authors declare no competing interests.

Author Contributions

- JAMM, ATC, and MAM conceived and designed the study;
- JAMM and CMP conducted the field study and carried out laboratory analyses;
- JAMM conducted statistical analyses and led manuscript writing;
- BNS ran model simulations and contributed to manuscript writing.

All authors actively revised manuscript drafts and gave final approval for publication.

Data Accessibility

Data collected for this study are archived online with the Environmental Data Initiative (https://doi.org/10.6073/pasta/e611de3fe6cd24c8666df91f45cb89b7). Model code is available on Zenodo (https://doi.org/10.5281/zenodo.3564527).

References

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., & Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of visualized experiments: JoVE, 81, 50961. doi: 10.3791/50961

Bradshaw, C. J. A. & Warkentin, I.G. (2015). Global estimates of boreal forest carbon stocks and flux. Global and Planetary Change, 128, 24-30. doi: 10.1016/j.gloplacha.2015.02.004

Brzostek, E. R., Dragoni, D., Brown, Z. A., & Phillips, R. P. (2015). Mycorrhizal type determines the magnitude and direction of root-induced changes in decomposition in a temperate forest. New Phytologist, 206(4), 1274-1282. doi: 10.1111/nph.13303

Buchkowski, R. W., Bradford, M. A., Grandy, A. S., Schmitz, O. J., & Wieder, W. R. (2017). Applying population and community ecology theory to advance understanding of belowground biogeochemistry. Ecology Letters, 20(2), 231-245. doi:10.1111/ele.12712

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., … Lindahl, B. D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. Science, 339(6127), 1615-1618. doi: 10.1126/science.1231923

Crow, S. E., Lajtha, K., Bowden, R. D., Yano, Y., Brant, J. B., Caldwell, B. A., & Sulzman, E. W. (2009). Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. Forest Ecology and Management, 258(10), 2224-2232. doi: 10.1016/j.foreco.2009.01.014

Crowther T. W., Todd-Brown, K. E. O., Rowe, C. W., Wieder, W. R., Carey, J. C., Machmuller, M. B., Snoek, L. B., … Bradford, M. A. (2016). Quantifying global soil carbon losses in response to warming. Nature, 540(7631), 104-108. doi: 10.1038/nature20150

DeAngelis, K. M., Brodie, E. L., DeSantis, T. Z., Andersen, G. L., Lindow, S. E., & Firestone, M. K. (2009). Selective progressive response of soil microbial community to wild oat roots. ISME Journal, 3(2), 168-178. doi: 10.1038/ismej.2008.103

Drake, J. E., Darby, B. A., Giasson, M. A., Kramer, M. A., Phillips, R. P., & Finzi, A. C. (2013). Stoichiometry constrains microbial response to root exudation-insights from a model and a field experiment in a temperate forest. Biogeosciences, 10(2), 821-838. doi: 10.5194/bg-10-821-2013

Finzi, A. C., Abramoff, R. Z., Spiller, K. S., Brzostek, E. R., Darby, B. A., Kramer, M. A., & Phillips, R. P. (2015) Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. Global Change Biology. 21(5), 2082-2094. doi: 10.1111/gcb.12816.

Franzluebbers, A. J. (1999) Potential C and N mineralization and microbial biomass from intact and increasingly disturbed soils of varying texture. Soil Biology and Biochemistry, 31(8), 1083-1090. doi: 10.1016/S0038-0717(99)00022-X

Haddix, M. L., Paul, E. A., & Cotrufo, M. F. (2016). Dual, differential isotope labeling shows the preferential movement of simple plant constituents into mineral-bonded soil organic matter. Global Change Biology, 22(6), 2301-2312. doi: 10.1111/gcb.13237

Hanson, P., Riggs, J., Dorrance, C., & Hook, L. (2011). SPRUCE environmental monitoring data: 2010–2014. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN. URL http://mnspruce.ornl.gov/. [accessed 29 September 2016].

Hartley, I. P., Hartley, I. P., Garnett, M. H., Sommerkorn, M., Hopkins, D. W., Fletcher, B. J., Sloan, V. L., Phoenix, G. K., & Wookey, P. A. (2012). A potential loss of carbon associated with greater plant growth in the European Arctic. Nature Climate Change, 2(12), 875-879. doi: 10.1038/nclimate1575.

Hicks Pries, C. E. et al. (2018). Root litter decomposition slows with soil depth. Soil Biology and Biochemistry, 125, 103-114. doi: 10.1016/j.soilbio.2018.07.002.

Holz, M., Aurangojeb, M., Kasimir, Å., Boeckx, P., Kuzyakov, Y., Klemedtsson, L., & Rütting, T. (2016). Gross nitrogen dynamics in the mycorrhizosphere of an organic forest soil. Ecosystems, 19(2), 284-295. doi: 10.1007/s10021-015-9931-4

Jenkinson, D. & Rayner, J. (1977). The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil Science, 123(5), 298-305. doi: 10.1097/00010694-197705000-00005

Jobbágy, E. G. & Jackson, R. B. (2000). The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecological Applications, 10(2), 423-436. doi: 10.1890/1051-0761(2000)010[0423:TVDOSO]2.0.CO;2

570 Johnson, D., Leake, J. R., & Read, D. J. (2001). Novel in-growth core system enables functional
571 studies of grassland mycorrhizal mycelial networks. New Phytologist, 152(3), 555-562.
572 doi: 10.1046/j.0028-646X.2001.00273.x
573 Keiluweit, M., Bougoure, J. J., Nico, P. S., Pett-Ridge, J., Weber, P. K., & Kleber, M. (2015).
574 Mineral protection of soil carbon counteracted by root exudates. Nature Climate Change,
575 5(6), 588-595. doi: 10.1038/nclimate2580
576 Kolka, R. K., Grigal, D. F., Nater, E. A., & Verry, E. S. (2001). Hydrologic cycling of mercury
577 and organic carbon in a forested upland-bog watershed. Soil Science Society of America
578 Journal, 65(3), 897-905. doi: 10.2136/sssaj2001.653897x
579 Kuzyakov, Y. (2010). Priming effects: Interactions between living and dead organic matter. Soil
580 Biology & Biochemistry, 42(9), 1363-1371. doi: 10.1016/j.soilbio.2010.04.003
581 Langley, J. A., Chapman, S. K., & Hungate, B. A. (2006). Ectomycorrhizal colonization slows
582 root decomposition: the post-mortem fungal legacy. Ecology Letters, 9(8), 955-959. doi:
583 10.1111/j.1461-0248.2006.00948.x
584 Lawrence, C. R., Neff, J. C., & Schimel, J. P. (2009). Does adding microbial mechanisms of
585 decomposition improve soil organic matter models? A comparison of four models using
586 data from a pulsed rewetting experiment. Soil Biology & Biochemistry, 41(9), 1923-
587 1934. doi: 10.1016/j.soilbio.2009.06.016
588 Li, X., Zhu, J., Lange, H., and Han, S. (2012). A modified ingrowth core method for measuring
589 fine root production, mortality and decomposition in forests. Tree Physiology, 33(1), 18-
590 25. doi: 10.1093/treephys/tps124
591 Lindahl, B. D., de Boer, W., & Finlay, R. D. (2010). Disruption of root carbon transport into
592 forest humus stimulates fungal opportunists at the expense of mycorrhizal fungi. ISME
593 Journal, 4(7), 872-881. doi: 10.1038/ismej.2010.19
594 Lobet, G. & Draye, X. (2013). Novel scanning procedure enabling the vectorization of entire
595 rhizotron-grown root systems. Plant Methods, 9(1), 1. doi: 10.1186/1746-4811-9-1
596 Lou, Y., Clay, S. A., Davis, A. S., Dille, A., Felix, J., Ramirez, A. H. M., Sprague, C. L., &
597 Yannarell, A. C. (2014). An affinity-effect relationship for microbial communities in
598 plant-soil feedback loops. Microbial Ecology, 67(4), 866-876. doi: 10.1007/s00248-013-
599 0349-2

Lynch, D. J., Matamala, R., Iversen, C. M., Norby, R. J., & Gonzalez-Meler, M. A. (2013). Stored carbon partly fuels fine-root respiration but is not used for production of new fine roots. New Phytologist, 199(2), 420-430. doi: 10.1111/nph.12290

McCormack, M. L., Dickie, A. D., Eissenstat, M., Fahey, T. J., Fernandez, C. W., Guo, D., Helmisaari, H. S., … Jackson, R. B. (2015). Redefining fine roots improves understanding of below-ground contributions to terrestrial biosphere processes. New Phytologist, 207(3), 505-518. doi: 10.1111/nph.13363

Meier, I. C., Finzi, A. C., & Phillips, R. P. (2017). Root exudates increase N availability by stimulating microbial turnover of fast-cycling N pools. Soil Biology & Biochemistry, 106, 119-128. doi: 10.1016/j.soilbio.2016.12.004

Moore, J. A. M., Jiang, J., Patterson, C. M., Mayes, M. A., Wang, G., & Classen, A. T. (2015). Interactions among roots, mycorrhizas and free-living microbial communities differentially impact soil carbon processes. Journal of Ecology, 103(6), 1442-1453. doi: 10.1111/1365-2745.12484

Parton, W. J., Hartman, M., Ojima, D., & Schimel, D. (1998). DAYCENT and its land surface submodel: description and testing. Global and Planetary Change, 19(1-4), 35-48. doi: 10.1016/s0921-8181(98)00040-x

Parton, W. J., Stewart, J. W. B., & Cole, C. V. (1988). Dynamics of C, N, P, and S in grassland soils - a model. Biogeochemistry, 5(1), 109-131. doi: 10.1007/bf02180320

Phillips, R. P., Finzi, A. C., & Bernhardt, E. S. (2011). Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecology Letters, 14(2), 187-194. doi: 10.1111/j.1461-0248.2010.01570.x

Phillips, R. P., Meier, I. C., Bernhardt, E. S., Grandy, A. S., Wickings, K., & Finzi, A. C. (2012). Roots and fungi accelerate carbon and nitrogen cycling in forests exposed to elevated CO_2. Ecology Letters, 15(9), 1042-1049. doi: 10.1111/j.1461-0248.2012.01827.x

Post, W. M., Emanuel, W. R., Zinke, P. J., & Stangenberger, A. G. (1982). Soil carbon pools and world life zones. Nature, 298(5870), 156-159. doi: 10.1038/298156a0

R Core Team (2014). fBasics: Rmetrics - Markets and Basic Statistics. R package version 3011.87.

R Core Team (2014). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2012. ISBN 3-900051-07-0.

Rasse, D. P., Rumpel, C., & Dignac, M.-F. (2005). Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil, 269(1), 341-356. doi: 10.1007/s11104-004-0907-y

Rewcastle, K. E., Moore, J. A. M., Henning, J. A., Mayes, M. A., Patterson, C. M., Wang, G., Metcalfe, D. B., & Classen, A. T. 2019. Investigating drivers of microbial activity and respiration in a forested bog. Pedosphere In press

Schimel, J. (2013). Soil carbon: microbes and global carbon. Nature Climate Change 3(10):867-868. doi: 10.1038/nclimate2015

Schimel, J. P., & Weintraub, M. N. (2003). The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry, 35(4), 549-563. doi: 10.1016/s0038-0717(03)00015-4

Shi, S., Richardson, A. E., O'Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., Firestone, M. K., & Condron, L. M. (2011). Effects of selected root exudate components on soil bacterial communities. FEMS Microbiology Ecology, 77(3), 600-610. doi: 10.1111/j.1574-6941.2011.01150.x

Six, J., Bossuyt, H., Degryze, S., and Denef, K. (2004). A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil and Tillage Research, 79, 7-31. doi: 10.1016/j.still.2004.03.008

Sulman, B. N., Phillips, R. P., Oishi, A. C., Shevliakova, E., & Pacala, S. W. (2014). Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. Nature Climate Change, 4(12), 1099-1102. doi: 10.1038/nclimate2436

Sulman, B. N., Shevliakova, E., Brzostek, E. R., Kivlin, S. K., Malyshev, S., Menge, D. N. L., and Zhang, X. (2019). Diverse mycorrhizal associations enhance terrestrial C storage in a global model. Global Biogeochemical Cycles, 33(4), 501-523. doi: 10.1029/2018GB005973

Tang, J. Y. (2015). On the relationships between the Michaelis–Menten kinetics, reverse Michaelis–Menten kinetics, equilibrium chemistry approximation kinetics, and quadratic kinetics. Geoscientific Model Development, 8, 3823-3835. doi: 10.5194/gmd-8-3823-2015

660 Taylor, K. E., Stouffer, R. J., & Meehl, G. A. (2012). An Overview of CMIP5 and the
661 Experiment Design. Bulletin of the American Meteorological Society, 93(4), 485-498.
662 doi: 10.1175/bams-d-11-00094.1
663 Todd-Brown, K. E. O., Hopkins, F. M., Kivlin, S. N., Talbot, J. M., & Allison, S. D. (2012). A
664 framework for representing microbial decomposition in coupled climate models.
665 Biogeochemistry, 109(1-3), 19-33. doi: 10.1007/s10533-011-9635-6
666 Todd-Brown, K. E. O., Randerson, J. T., Post, W. M., Hoffman, F. M., Tarnocai, C., Schuur, E.
667 A. G., & Allison, S. D. (2013). Causes of variation in soil carbon simulations from
668 CMIP5 Earth system models and comparison with observations. Biogeosciences, 10(3),
669 1717-1736. doi: 10.5194/bg-10-1717-2013
670 Treseder, K. K., & Lennon, J. T. (2015). Fungal traits that drive ecosystem dynamics on land.
671 Microbiology and Molecular Biology Reviews, 79(2), 243-262. doi:
672 10.1128/MMBR.00001-15
673 Treseder, K. K., Balser, T. C., Bradford, M. A., Brodie, E. L., Dubinsky, E. A., Eviner, V. T.,
674 Hofmockel, K. S., … Waldrop, M. P. (2012). Integrating microbial ecology into
675 ecosystem models: challenges and priorities. Biogeochemistry, 109(1-3), 7-18. doi:
676 10.1007/s10533-011-9636-5
677 Vance, E., Brookes, P., & Jenkinson, D. (1987). An extraction method for measuring soil
678 microbial biomass C. Soil Biology & Biochemistry, 19(6), 703-707. doi: 10.1016/0038-
679 0717(87)90052-6
680 Vogt, K. A., Vogt, D. J., Palmiotto, P. A., Boon, P., O'Hara, J., & Asbjornsen, H. (1995). Review
681 of root dynamics in forest ecosystems grouped by climate, climatic forest type and
682 species. Plant and Soil, 187(2), 159-219. doi: 10.1007/bf00017088
683 Voroney, R., Brooks, P. C., & Beyaert, R. (2007). Soil microbial biomass C, N, P, and S. In: M.
684 R. Carter & E. G. Gregorich, (Eds.), Soil Sampling and Methods of Analysis, Second
685 Edition (pp. 637-652). Boca Raton, FL: CRC Press.
686 Wang, G., Jagadamma, S., Mayes, M. A., Schadt, C. W., Steinweg, J. M., Gu, L., & Post, W. M.
687 (2015). Microbial dormancy improves development and experimental validation of
688 ecosystem model. ISME Journal, 9(1), 226-237. doi: 10.1038/ismej.2014.120

689 Xu, S., Liu, L. L., & Sayer, E. J. (2013). Variability of above-ground litter inputs alters soil
690 physicochemical and biological processes: a meta-analysis of litterfall-manipulation
691 experiments. Biogeosciences, 10(11), 7423-7433. doi: 10.5194/bg-10-7423-2013
692 Xu, X., Schimel, J. P., Thornton, P. E., Song, X., Yuan, F., & Goswami, S. (2014). Substrate and
693 environmental controls on microbial assimilation of soil organic carbon: a framework for
694 Earth system models. Ecology Letters, 17(5), 547-555. doi: 10.1111/ele.12254
695 Yin, H., Li, Y., Xiao, J., Xu, Z., Cheng, X., & Liu, Q. (2013). Enhanced root exudation
696 stimulates soil nitrogen transformations in a subalpine coniferous forest under
697 experimental warming. Global Change Biology, 19(7), 2158-2167. doi:
698 10.1111/gcb.12161
699 Zak, D. R., & Kling, G. W. (2006). Microbial community composition and function across an
700 arctic tundra landscape. Ecology, 87(7), 1659-1670. doi: 10.1890/0012-
701 9658(2006)87[1659:mccafa]2.0.co;2

Figures

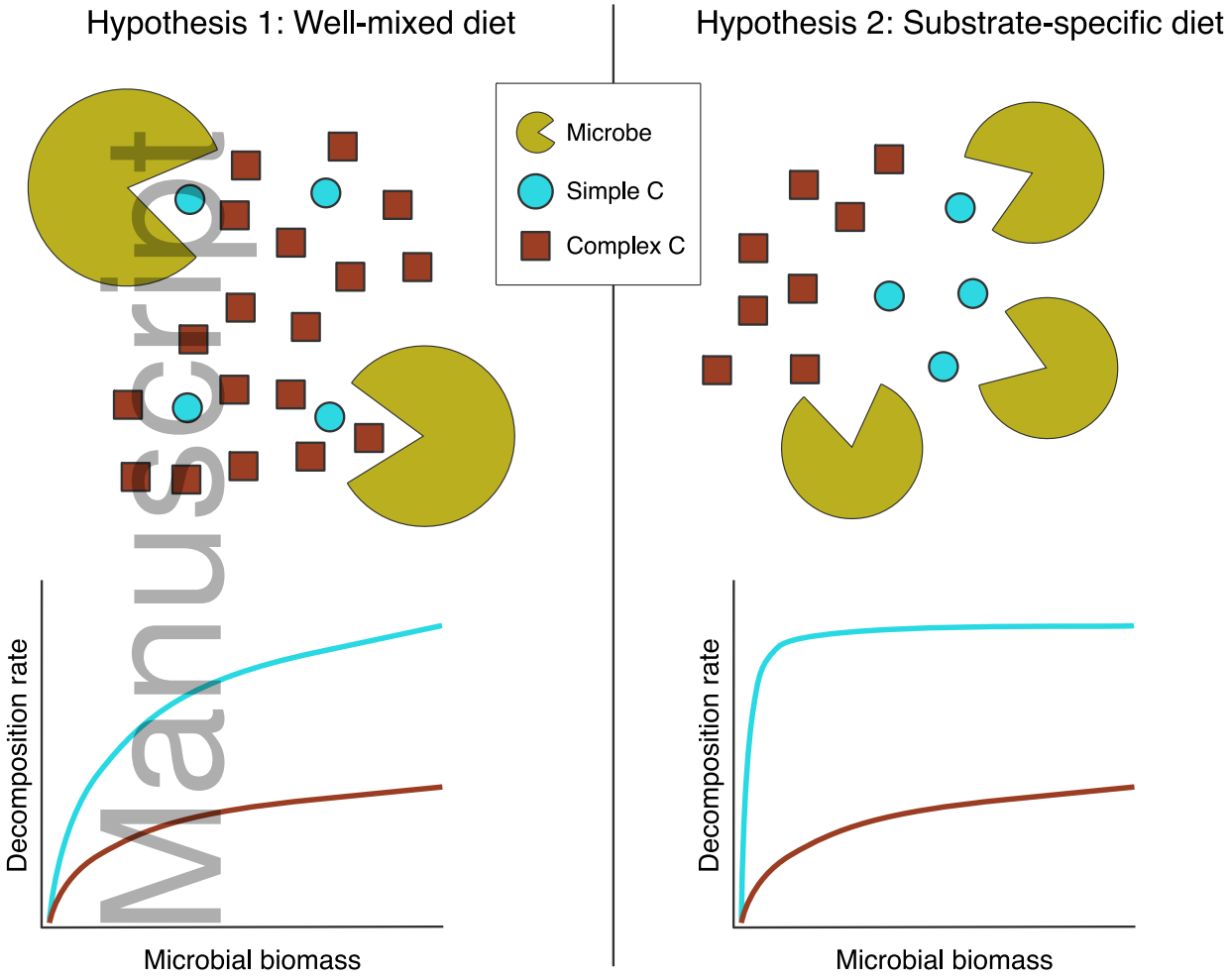


Figure 1: We simulated two hypothetical frameworks using the CORPSE model of C pools and flows. In Hypothesis 1, we structured CORPSE to allow microbial breakdown of a mixture of substrates with low substrate affinity. In Hypothesis 2, we structured CORPSE to restrict microbial access to particular substrate types with high substrate fidelity. The relationship between microbial biomass and substrate fidelity was expected to have consequences for the degree of saturation of decomposition rates with increasing microbial biomass.

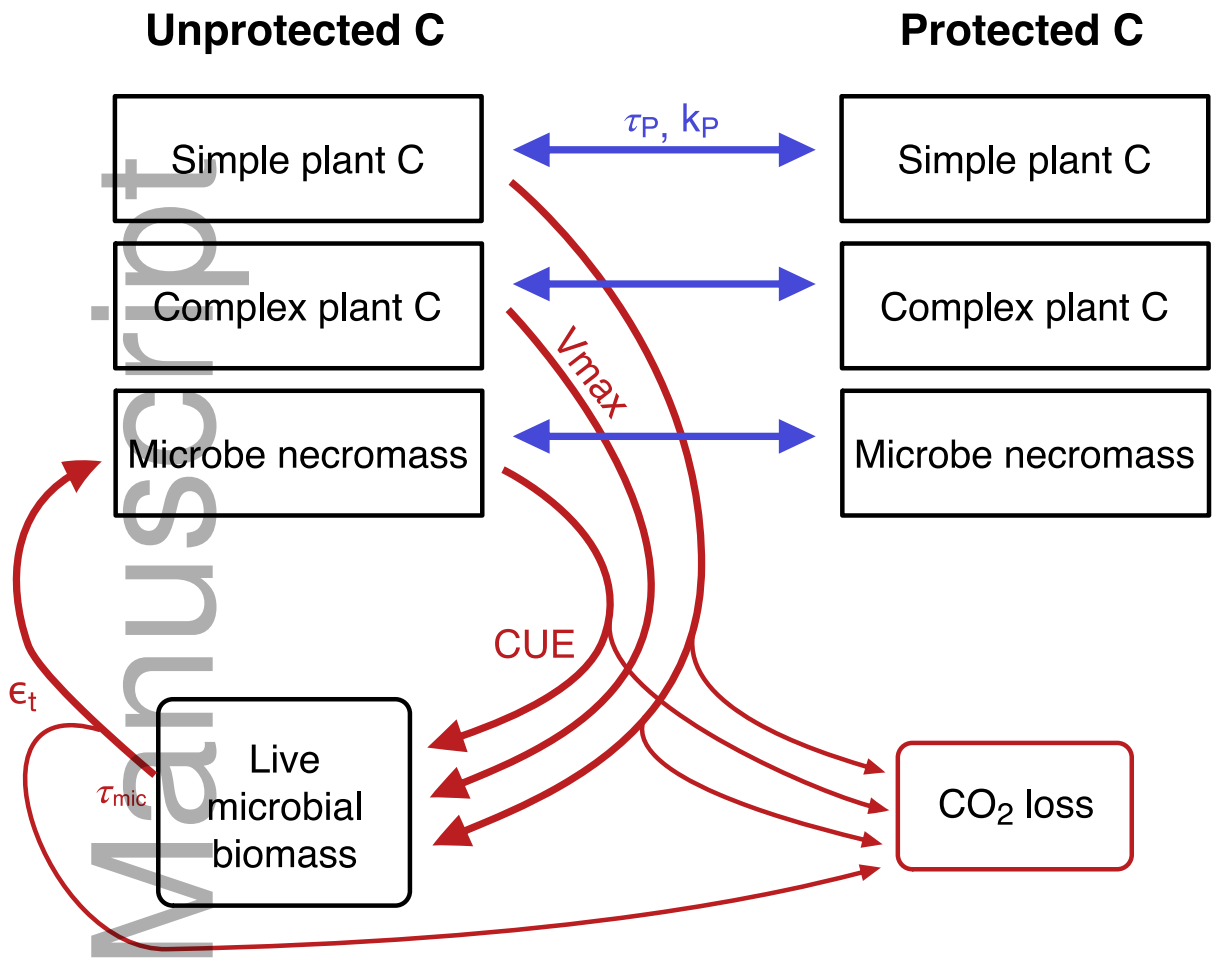


Figure 2: Carbon pools (boxes) and flows (arrows) in the CORPSE model. Plants assimilate carbon and transfer simple and complex C to microbes. Plant C and microbial necromass C can be unprotected and available for microbial uptake, or in a protected pool that is unavailable for microbial uptake. Flow of C between protected and unprotected pools occurs at a slow but constant rate. C flow into live microbial biomass contributes to growth of that pool, is released as CO_2, or contributes to the microbial necromass pool. Parameters that were modified to test the model structural hypotheses included microbial enzyme kinetics (k_P and V_{max}) and microbial C use efficiency (CUE). Our rhizosphere manipulation experiment used isotope tracers to track C flow from simple and complex pools through to CO_2 loss, or soil respiration. See the CORPSE Simulations section for a detailed model description and equations and Supplemental Table 2 for model parameters.

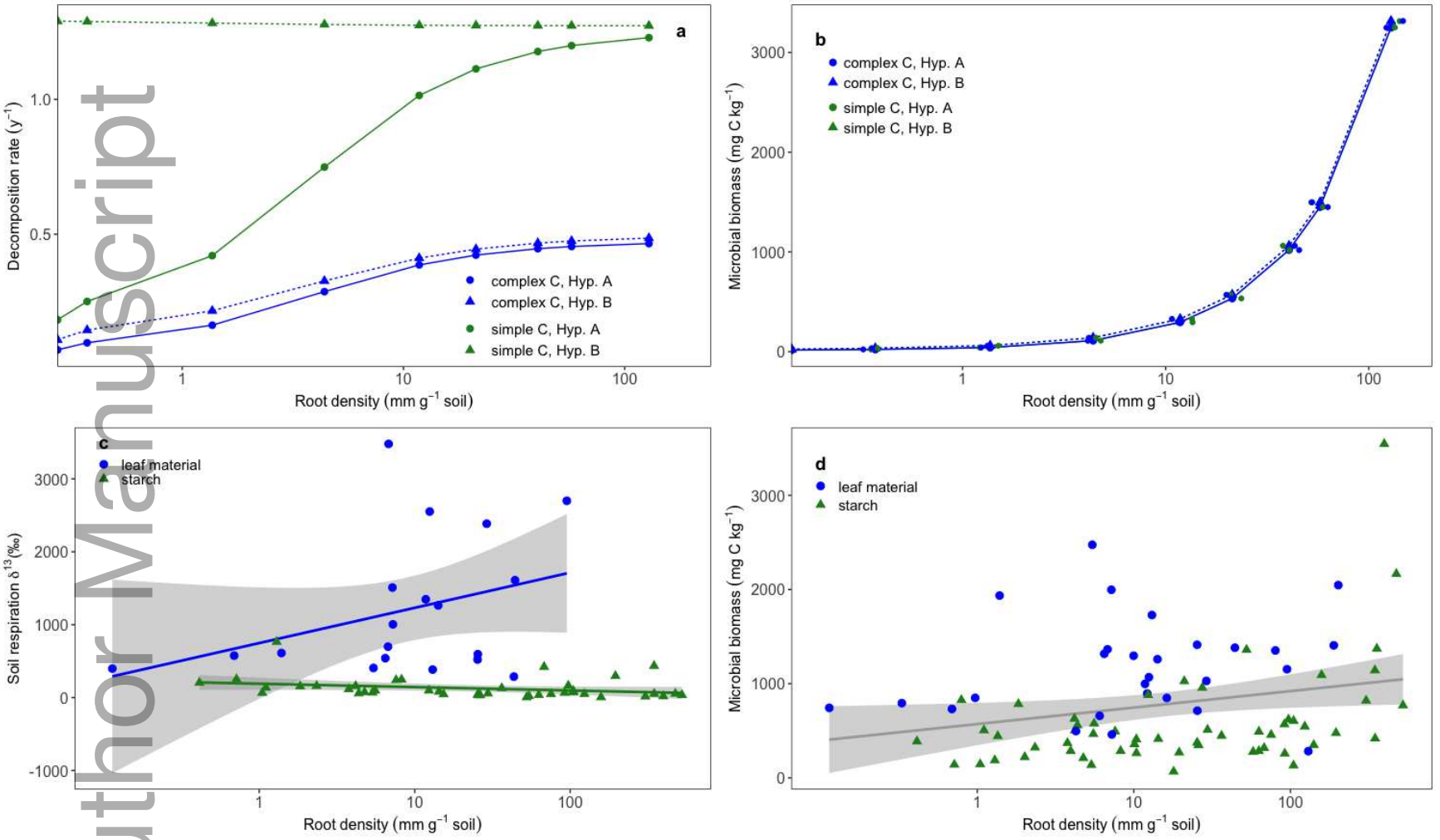
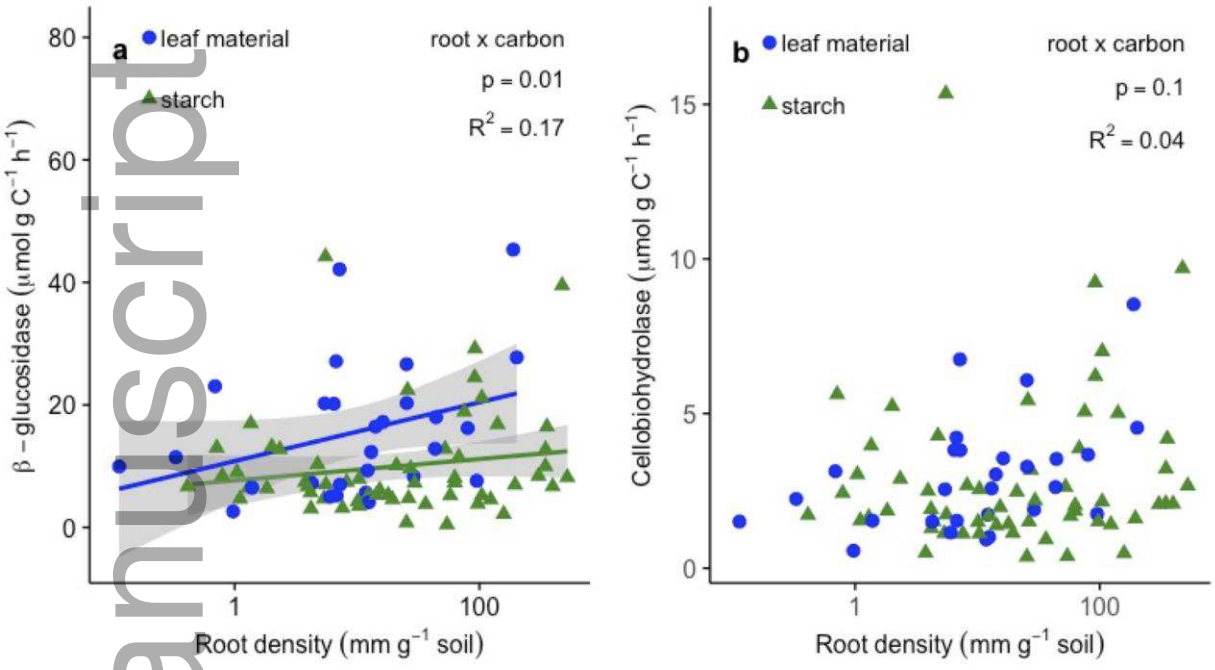


Figure 3: Relationships between root density, decomposition rate, and microbial biomass in model simulations (a, b), and between root density, soil respiration, and microbial biomass in the field experiment (c, d). The decomposition rate per unit mass for complex C (blue) and simple C (green) in CORSPE is shown relative to simulations with zero root exudation (i.e., plant simple C inputs) and as a

function of root density (a). Results from CORPSE simulations are shown for Hypothesis A (circles) and Hypothesis B (triangles). Simulated microbial biomass as a function of root density (b). ^{13}C-labeled leaf material (blue circles) and ^{13}C-labeled starch (green triangles) as a function of increasing root density (c). Microbial biomass as a function of increasing root length, for ^{13}C-labeled leaf material (blue circles) and ^{13}C-labeled starch (green triangles) (d).



732

733 Figure 4: The carbon-degrading enzymes ß-glucosidase (a) and cellobiohydrolase (b) as a
734 function of increasing root density. Enzyme activity was measured in soils amended with leaf
735 material (blue circles) and starch (green triangles).