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Stoichiometry constrains microbial response to root exudation – insights from a model and a field experiment in a temperate forest

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Abstract

Healthy plant roots release a wide range of chemicals into soils. This process, termed root exudation, is thought to increase the activity of microbes and the exo-enzymes they synthesize, leading to accelerated rates of carbon (C) mineralization and nutrient cycling in rhizosphere soils relative to bulk soils. The causal role of exudation, however, is difficult to isolate with in-situ observations, given the complex nature of the rhizosphere environment. We investigated the potential effects of root exudation on microbial and exo-enzyme activity using a theoretical model of decomposition and a field experiment, with a specific focus on the stoichiometric constraint of nitrogen (N) availability. The field experiment isolated the effect of exudation by pumping solutions of exudate mimics through microlysimeter “root simulators” into intact forest soils over two 50-day periods. Using a combined model-experiment approach, we tested two hypotheses: (1) *exudation alone is sufficient to stimulate microbial and exo-enzyme activity in rhizosphere soils*, and (2) *microbial response to C-exudates (carbohydrates and organic acids) is constrained by N-limitation*. Experimental delivery of exudate mimics containing C and N significantly increased microbial respiration, microbial biomass, and the activity of exo-enzymes that decompose labile components of soil organic matter (SOM, e.g., cellulose, amino sugars), while decreasing the activity of exo-enzymes that degrade recalcitrant SOM (e.g., polyphenols, lignin). However, delivery of C-only exudates had no effect on microbial biomass or overall exo-enzyme activity, and only increased microbial respiration. The theoretical decomposition model produced complementary results; the modeled microbial response to C-only exudates was constrained by limited N supply to support the synthesis of N-rich microbial biomass and exo-enzymes, while exuding C and N together elicited an increase in modeled microbial biomass, exo-enzyme activity, and decomposition. Thus, hypothesis (2) was supported, while hypothesis (1) was only supported when C and N compounds were exuded together. This study supports a cause-and-effect relationship between root exudation and enhanced microbial

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activity, and suggests that exudate stoichiometry is an important and underappreciated driver of microbial activity in rhizosphere soils.

1 Introduction

The transfer of carbon (C) from tree roots to soil is important but poorly understood relative to other processes in the terrestrial C cycle (Grayston et al., 1997; Jones et al., 2004). Healthy and intact roots release a wide variety of chemicals into the surrounding soil (i.e. the rhizosphere), a process termed exudation (Rovira, 1969). Exudation directly couples tree physiology with soil processes such as microbial physiology, decomposition, and nutrient cycling (Farrar et al., 2003). Exudation varies depending on tree species, mycorrhizal associations, and environmental factors affecting tree physiology such as atmospheric CO₂ concentration, temperature, and nutrient supply (Farrar et al., 2003; Phillips et al., 2009). It is possible that tree responses to global change factors will affect exudation and nutrient cycling in soils, and thus influence ecosystem-scale processes such as net primary production and soil C storage (e.g., Langley et al., 2009; Drake et al., 2011; Phillips et al., 2011).

Root exudation has a number of physiological and environmental functions. Plants use membrane-bound ATP-ase H⁺ pumps to create a negative membrane potential that aids in cation uptake; this membrane potential also drives negatively-charged compounds out of root cells, including many organic acids such as citrate⁻³, malate⁻², and oxalate⁻² (Farrar et al., 2003; Jones et al., 2004). Exudation of these organic acids can mobilize nutrients from soil cation exchange sites, particularly phosphorus in soils with low pH, and help plants avoid aluminum toxicity through chelation (Basu et al., 1994; Jones and Darrah, 1994a; Jones, 1998; Ryan et al., 2001; Dakora and Phillips, 2002). Some exuded chemicals also act as signaling molecules or toxic allelochemicals (Grayston et al., 1997; Bertin et al., 2003; Somers et al., 2004). While organic acids and carbohydrates are the dominant chemicals found in root exudates, the few studies that have measured exudate composition have also found a substantial net

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5 efflux of amino acids (Bowen, 1969; Rovira, 1969; Smith, 1976; Grayston et al., 1997; Bertin et al., 2003; but see Jones et al., 2009). While bacterial biosensor studies have confirmed that soil microbes access amino acids exuded from roots, the effect of nitrogen (N) compounds in root exudates on microbial activity and biogeochemical fluxes is largely unknown (Jaeger et al., 1999; Vilchez et al., 2000; Espinosa-Urgel and Ramos, 2001).

Here, we study the effects of root exudates on nutrient availability through their effect on microbial activities that enhance the decomposition of soil organic C and N (SOC and SON). Microbes rapidly absorb exudates and use them for metabolism and exo-enzyme production (Jones et al., 1996; Jones, 1998). Thus, exudation has been invoked as the mechanism causing increased microbial and enzymatic activity in rhizosphere soils relative to bulk soils (e.g., Dakora and Phillips, 2002; Paterson, 2003), and increased exudation under elevated atmospheric concentrations of CO₂ has been used to explain increases in gross N mineralization rates and whole-ecosystem soil N uptake (Norby et al., 1987; Drake et al., 2011; Phillips et al., 2011). While it is logical that increased exudation would increase the activity of soil heterotrophs, there have been few direct tests of the cause-and-effect nature of this relationship (but see Kuzyakov et al., 2007; Shi et al., 2011). Furthermore, rhizospheres have high physical and biotic complexity (Dakora and Phillips, 2002; Cardon and Gage, 2006), suggesting that complex interactions between exudates, organic matter, and soil microbes are likely to occur. Thus, while many authors have invoked exudation as a mechanism to explain higher rates of decomposition and higher nutrient availability in rhizosphere soils, we still know relatively little about the direct role of exudates per se on microbial activity and soil nutrient cycling.

25 In this study, we investigated the effects of root exudation on the activity of soil microbes and exo-enzymes using a theoretical model of decomposition and a field experiment simulating root exudation. In the field experiment, we pumped solutions of chemicals often found in root exudates (“exudate mimics”) through microlysimeter root simulators into intact forest soils over two 50-day periods. Using this combined

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model-experiment approach, we tested the following two hypotheses: (1) *if exudation alone is sufficient to stimulate microbial activity in rhizosphere soils, then the addition of exudate mimics will lead to higher microbial biomass, increased exo-enzyme activities, and higher rates of C-mineralization relative to bulk soils*, and (2) *if the microbial response to C-exudates (carbohydrates and organic acids) is constrained by microbial N-limitation, then exuding C and N together will elicit a larger rhizosphere response relative to exuding C alone*.

2 Methods

2.1 Theoretical modeling of decomposition

We used an existing modeling framework to explore how decomposition may respond to the delivery of root exudates (Schimel and Weintraub, 2003). This theoretical model operates on the scale of a gram of soil with an hourly time-step and has no environmental drivers. The purpose of the model was to explore theoretical linkages between exo-enzymatic depolymerization of soil organic matter (SOM), microbial physiology, exudation, and stoichiometry (C:N ratio), not to quantitatively predict decomposition in a particular study location. This model explicitly simulates microbial production of exo-enzymes that depolymerize complex and insoluble SOM into soluble monomers that are available for microbial uptake (Fig. 1a). Thus, this model incorporates mechanisms related to microbial physiology and exo-enzyme activity that are known to control decomposition in soils (Schimel and Bennett, 2004; Blagodatsky et al., 2010), but differs from common decomposition models that assume SOM pools decompose at a characteristic first-order rate, modified by empirical temperature and moisture functions (e.g. the CENTURY and RothC models: Parton et al., 1988, 1993; Coleman et al., 1997; Zimmermann et al., 2007). The decomposition model is described in detail in Appendix A.

In brief, the model is composed of five pools connected by fluxes (Fig. 1a). SOM is depolymerized into dissolved organic matter (DOM) depending on the quantity of

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5 exo-enzymes in a reverse Michaelis–Menten manner as in Schimel and Weintraub (2003). DOM is taken up by microbial biomass according to Michaelis–Menten kinetics as in Allison et al. (2010) and microbes use these resources to fuel exo-enzyme and biomass production according to first-order equations, each of which has a respira-
10 tory cost determined by a substrate-use-efficiency (SUE) parameter. When N demand exceeds N supply (i.e. under N-limitation), the surplus C is mineralized as overflow metabolism; when C demand exceeds C supply (i.e. under C-limitation), the surplus N is mineralized (as in Schimel and Weintraub, 2003). Microbes and enzymes turn over with first-order kinetics and the resulting material re-enters SOM and DOM pools (as
15 in Schimel and Weintraub, 2003). We made four changes to the model of Schimel and Weintraub (2003): we included Michaelis–Menten kinetics for microbial uptake of DOM, fixed an error in the equation governing inorganic N immobilization, introduced a flux by which inorganic N was removed from the model, and made enzyme production proportional to microbial biomass as in Allison et al. (2010). These changes are discussed in detail in Appendix A.

Model simulations were used to test the theoretical underpinnings of the two hypotheses and motivate the design of the field experiments that are described below (Fig. 1b). We explored how the modeled response to exudate pulses varied with the stoichiometry of the system; that is, we ran the model with varying C : N ratios of exu-
20 dates, SOM, exo-enzymes, and microbes (Fig. 2). We also performed a sensitivity analysis to determine which parameters most affected the response of net mineralization and SOM decomposition to exudate pulses (Table 1). Parameter values were taken from the literature (Schimel and Weintraub, 2003; Allison et al., 2010), and we assessed model sensitivity to halving and doubling these values (Table 1). In our simu-
25 lations, however, SOM and exo-enzyme C : N ratios were limited to ranges of 10 to 30 (Batjes, 1996; Aitkenhead and McDowell, 2000), and 2 to 4, respectively (Sterner and Elser, 2002). For each parameter set, we quantified the additional N depolymerized and mineralized from SOM because of an exudate pulse by integrating the response variable after the pulse relative to a model run with identical parameters but no exudate

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pulse. Sensitivity was calculated as in Allison et al. (2010) in a way that normalizes the change in output relative to the size of the change in parameterization, as:

$$\text{Sensitivity} = \frac{|\log| \text{high output} | - \log| \text{low output} ||}{|\log| \text{high parameter value} | - \log| \text{low parameter value} ||} \quad (1)$$

2.2 Site of field experiments

5 After the model provided theoretical support for the two hypotheses (Sect. 3.1, below), we conducted experimental tests using a series of field experiments. These experiments were conducted in the Prospect Hill tract of Harvard Forest, a Long Term Ecological Research (LTER) site in Central Massachusetts, USA. This site was comprised of a mixed deciduous forest primarily dominated by red oak (*Quercus rubra* L.)
10 and red maple (*Acer rubrum* L.) recovering from a hurricane disturbance in 1938. The soil was derived from glacial till and was a well-aerated fine sandy loam, classified as a Typic Dystrochrept (Borken et al., 2006). Long-term meteorological observations at this site (1964–2010) recorded a mean annual temperature of 7.5 °C and evenly distributed precipitation throughout the year with a mean annual value of 1120 mm (Boose and Gould, 1999; Boose, 2001). This particular forest location has been the site of extensive research regarding the controls of CO₂ and other trace gas fluxes from soil (Davidson et al., 1998; Savage and Davidson, 2001; Borken et al., 2003, 2006; Davidson et al., 2006; Savage et al., 2009). Net ecosystem exchange (NEE) of CO₂ has been measured since 1991 at the nearby environmental measurement site; these data
15 were used to determine the timing of exudation treatments (Munger and Wofsy, 1999; Urbanski et al., 2007). We scheduled the exudation treatments to occur during periods with high rates of net CO₂ uptake, with the assumption that root exudation is high during periods of high photosynthetic C-uptake (Dilkes et al., 2004; Phillips and Fahey, 2005).

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2.3 Treatments mimicking root exudation

We simulated root exudation in the field by pumping solutions containing compounds typically found in root exudates (hereafter, “exudate mimics”) through microlysimeters inserted vertically into the top 10 cm of mineral soil (Rhizon soil solution samplers, Rhizosphere Research Products, Wageningen, Netherlands; see lab study by Kuzyakov et al., 2007). The cylindrical microlysimeters were 10 cm long and 2.5 mm in diameter with a mean pore size of 0.15 μm . Solutions were pumped through the microlysimeters using peristaltic pumps (Model 205U, Watson-Marlow, Wilmington, Massachusetts, USA) and 0.25 mm-diameter tubing (Marprene, Watson-Marlow) daily from 11:00–11:15 LT and 14:00–14:15 LT using automatic timers (Model DT620CL, Intermatic Inc., Spring Grove, Illinois, USA) at a flow rate of 0.06 mL min⁻¹, which delivered an average of 1.8 mL of solution microlysimeter⁻¹ d⁻¹. The exudate delivery system was originally designed and tested at the Duke Free Air CO₂ Enrichment site (R. P. Phillips and E. S. Bernhardt, personal communication). We elected to use two daily pulses because our system of pumps and tubing could not operate accurately when run continuously at the extremely low flow rates that would be required to avoid large water additions. We think the main advantage of the field-based microlysimeter approach is the delivery of small quantities of exudate mimics to intact soils that are subject to the full suite of biotic and abiotic factors that occur in the field. This approach differs from most experiments that have added compounds to soil, often in large single doses or to disturbed soils in the lab (e.g., De Nobili et al., 2001; Vance and Chapin, 2001; Fontaine et al., 2004; Brant et al., 2006).

This automated system was used to deliver solutions to forest soils in four treatments: a C-only addition, a C + N addition, a water control, and a disturbance control. The C-only treatment consisted of a 500 mg Cl⁻¹ solution comprised of 75 % organic acids and 25 % carbohydrates. A stock solution for this treatment was made by dissolving 1.25 g citric acid, 2.47 g oxalic acid, 1.13 g fumaric acid, 1.35 g malonic acid, and 1.56 g glucose in one liter of water; this stock solution was diluted 1 : 10 before

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use. These compounds reflect our best estimate of exudate chemistry from the relatively few literature studies on this subject (Bowen, 1969; Rovira, 1969; Smith, 1976; Bertin et al., 2003; Jones et al., 2009). The C + N treatment consisted of the same delivery solution used for the C-only treatment, plus 50 mgNL⁻¹ as NH₄Cl, yielding a solution with a C : N ratio of 10. We used NH₄Cl instead of an organic N source to test specifically whether N availability constrains microbial responses to C exudates. We acknowledge that amino acids rather than NH₄⁺ are exuded from roots, but our objective was to test the effect of exudate stoichiometry on rhizosphere processes; had we used an organic N compound we could not meaningfully compare the results of the C-only treatments to those containing both C and N because C content would have been confounded with N content. The water control consisted of the same deionized water used to make the C-only and C + N solutions. The disturbance control treatment consisted of installing microlysimeters in the soil without addition of water or exudate mimics. The water, C-only, and C + N treatment solutions were replaced weekly to minimize the effects of contamination. These concentrations and the delivery flow rate were chosen to mimic rates of exudation observed for four temperate tree species in this region (Brzostek et al., 2012). We delivered these solutions to ten microlysimeters per treatment ($n = 10$).

Exudates were delivered from 6 June to 25 July 2011 (50 days) and from 8 August to 26 September 2011 (50 days). The first experiment was timed to capture the early summer period after leaf expansion, and the second experiment was timed to capture the mid-late summer period prior to leaf coloration (Richardson and O'Keefe, 2009). Net ecosystem production is typically strongly positive during these periods, indicating net ecosystem C uptake (Fig. B1).

2.4 Soil sampling

We collected soil in contact with each microlysimeter on 25 July and 26 September 2011 using sharpened aluminum tubes measuring 13.5 mm in diameter and 10 cm in length. A test injection with blue dye demonstrated that a 500 μ L injection twice a day

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delivered the solution to a cylinder of soil ~5 mm in radius from the microlysimeter (R. P. Phillips and A. C. Finzi, personal observation), so the small soil cores adequately sampled the influenced soil with minimal contamination of bulk soil. Each core yielded ~ 15 g of soil. The soil samples was transported to Boston University in a cooler, stored overnight at 4 °C, and processed the following day (Sect. 2.5). Soils were homogenized and roots were removed by hand.

2.5 Soil process rates

We measured a number of processes related to the activity of soil microbes to assess the degree to which the exudation treatments influenced heterotrophic activity. We measured microbial respiration, microbial biomass, proteolytic rates, and extracellular enzyme activities.

The rate of CO₂ production by heterotrophs (hereafter, “microbial respiration”) for each soil sample was measured in the lab using short-term incubations; 2 g of soil was placed in a septum-sealed 500 mL glass jar and incubated at lab temperature (~ 23 °C). Three headspace samples (10 mL) were taken at 2 h intervals and injected into an infrared gas analyzer (Model EGM-4; PP-Systems, Amesbury, Massachusetts, USA) to determine the concentration of CO₂. These concentrations increased at a linear rate for all samples (minimum $r^2 = 0.93$, average $r^2 = 0.98$). The rate of CO₂ production per g dry soil was calculated from these slopes, the jar volume, soil mass, and soil gravimetric water content.

Microbial biomass was measured for each sample using the substrate-induced respiration (SIR) method for both sampling dates (25 July and 26 September 2011) and the chloroform-fumigation extraction (CFE) method for the second sampling date only (26 September 2011). The SIR method was performed just after the microbial respiration measurements described above by adding a glucose solution to each soil jar to achieve an addition of 2 mL and 20 mg glucose g soil⁻¹ as in Harden et al. (1993). The glucose solution was added drop-by-drop over the surface of the soil and the jars were shaken by hand to achieve a well-mixed delivery of glucose. After waiting one hour,

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the rate of CO₂ production was measured using three headspace samples taken at hourly intervals using the same procedure as described above for microbial respiration. The rate of CO₂ production following glucose addition was linear for all samples (minimum $r^2 = 0.98$, average $r^2 = 0.99$). Maximum rates of SIR were converted to microbial biomass using a previously published equation (Anderson and Domsch, 1978; Phillips et al., 2011). Microbial biomass N was measured using the CFE procedure assuming an extraction efficiency (k_n) value of 0.68 (Gallardo and Schlesinger, 1990).

The activity of soil proteolytic enzymes was measured as the gross rate of increase in free amino acids (OPAME method of Jones et al., 2002) over a 4 h incubation using 2 g of soil as previously described (Watanabe and Hayano, 1995; Lipson et al., 1999; Brzostek and Finzi, 2011). This conversion of organic N polymers into soluble amino acid monomers is hereafter referred to as proteolysis; this process has been characterized as a rate-limiting step for the decomposition of SOM (Schimel and Bennett, 2004).

We measured the activity of extracellular enzymes related to phosphorus mineralization (acid phosphatase, herein abbreviated as AP) and the decomposition of amino-sugars (β -1,4-N-acetyl-glucosaminidase; NAG), cellulose (β -1,4-glucosidase; BG), and lignin (peroxidase and phenol oxidase; PerO_x and PhenO_x, respectively; Finzi et al., 2006). The activity of these exo-enzymes was measured by mixing 1 g of soil with 100 mL of 50 mM sodium acetate buffer adjusted to pH = 5.0. The samples were continuously stirred and twenty-four 200 μ L aliquots of the suspension were transferred to 96-well microplates; exo-enzyme activities were subsequently measured exactly as described previously (Finzi et al., 2006) using 96-well plate readers (VersaMax and SpectraMax Gemini XS, Molecular Devices, Sunnydale, California, USA) and expressed as nmol substrate utilized g⁻¹ drysoil h⁻¹. Lignolytic enzymes (PerO_x and PhenO_x) were measured colorimetrically using L-dihydroxyphenylalanine (L-DOPA) substrate with a 4 h incubation; all other enzymes were measured fluorimetrically using substrates linked to a fluorescent tag (4-methylumbelliferone) with a 2 h incubation.

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We used the decomposition model (described above, Sect. 2.1) to predict the expected response of microbial biomass to the exudation treatments. That is, we evaluated if the model predictions for a 50-day experiment with twice-daily exudate pulses agreed with the experimental results. The model was parameterized using established values for all parameters (Table 1; Schimel and Weintraub, 2003; Allison et al., 2010) and two exudate pulses were delivered per day, which matched the field experiments. Model runs were performed with no exudate treatment, a C-only treatment, or treatment with C and N together at a C : N ratio of 10; these simulations were developed to match the experimental treatments.

2.6 Statistical analyses

The exudation field experiments consisted of a completely randomized design with ten replicates for each of four categories of treatment (disturbance control, water, C-only, and C + N). Results were analyzed in the GLM procedure of the SAS system (SAS 9.1; SAS Institute Inc., Cary, North Carolina, USA). Treatment was included as a fixed effect. As there were two separate experiments, the mean of each experiment and an experiment \times treatment interaction term were included as random effects. When the main effect of treatment was statistically significant, statistical comparisons of individual exudation treatments were performed using Duncan's multiple range post-hoc test, which reduces the number of individual comparisons and thus partially controls the experiment-wise error rate (Duncan, 1955; Littell et al., 2002). The assumptions that model residuals were normally distributed with mean zero and constant variance were met in all cases; no transformations were necessary.

In addition to analyzing the data for each enzyme activity assay as above, we summarized the effects of exudation treatments across all enzymes using meta-analysis (MetaWin v.2.1, Rosenberg et al., 2000). While meta-analysis is frequently used to synthesize data from multiple experiments (e.g., Rustad et al., 2001; Ainsworth and Long, 2005) it can also be used to synthesize treatment effects on soil enzymes within single studies (Hedges and Olkin, 1985; Saiya-Cork et al., 2002). We calculated the

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natural log of the response ratio (i.e., $\ln(\text{treatment value}/\text{disturbance control value})$) and the variance around this ratio for each enzyme activity measurement. We calculated the effect size and bias-corrected bootstrapped 95 % confidence intervals (CI's) for the mean response of soil enzyme activities to water, C-only, and C + N exudation treatments. For this analysis, the enzymes were grouped into those that decompose labile substrates (proteolytic enzymes, AP, NAG, and BG) and those that decompose recalcitrant substrates (PerO_x and PhenO_x ; Table 2). Positive response ratios indicate that exudation treatments increased enzyme activities relative to unamended disturbance controls.

3 Results

3.1 Modeling

Microbial respiration increased in two distinct phases following a modeled exudate pulse of DOC and DON; there was an abrupt increase in respiration associated with the construction of new microbial biomass (Fig. 2e–h) and exo-enzymes (Fig. 2i–l), followed by a small but sustained increase in the maintenance respiration of microbial biomass (Fig. 2a–d). The increase in the exo-enzyme pool size following an exudate pulse affected the decomposition of SOM and N mineralization in three phases. Initially, there was an abrupt spike of N mineralization when the DON additions briefly pushed microbes into a state of C-limitation (e.g. red line in Fig. 2m, black line in Fig. 2o). This initial phase was not observed in all of the model runs. Next, N mineralization dropped to zero while the microbes were utilizing all available N to produce biomass and exo-enzymes (Fig. 2m–p). Thus, modeled exudation briefly stimulated microbial uptake of organic and inorganic N and reduced the N that would be available to plant roots. Finally, the microbes returned to a state of C limitation and began mineralizing N. During this period, rates of N mineralization and the depolymerization of SON into DON were higher than the rates preceding the pulse (Fig. 2m–p). Thus, the model predicts that an

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exudate pulse can theoretically increase the amount of organic and inorganic N available for root uptake, after a brief period of microbial immobilization. In the long-term, all pools and fluxes eventually returned to pre-exudate equilibrium values.

While this pattern was generally consistent across model runs, the stoichiometry of the system affected the microbial and enzymatic response to exudate delivery. The stoichiometry of the exudate pulse itself had the largest effect on microbial and exo-enzyme activity (Fig. 2a, e, i, m); exuding N along with C strongly stimulated the microbial and exo-enzymatic response. A larger response in microbial biomass and exo-enzyme pools was observed when the N-supply potential of the system was high (i.e., a low soil C : N ratio and low exudate C : N) and when microbial N demand was low (i.e., with high microbial C : N and high enzyme C : N ratios). Varying microbial C : N also affected the modeled response to an exudate pulse (Fig. 2c, g, k, o), while varying exo-enzyme C : N only influenced N mineralization (Fig. 2d, h, l, p).

In the sensitivity analysis of kinetic and stoichiometric model parameters, we found that the rate of exo-enzyme loss (K_L) as well as the size and C : N ratio of the exudate pulse had the greatest effects on the quantity of N mineralized following an exudate pulse (Table 1). In particular, exudate pulse size and C : N ratio influenced the amount of additional exo-enzymes synthesized, whereas the rate of enzyme loss determined how long the extra enzymes were active. When all possible combinations of parameter sets were varied (i.e., all values in Table 1), N mineralization following an exudate pulse increased as the quantity of N-exudation increased (Fig. 3). This relationship was above 1 : 1 for most model parameter sets, indicating a net increase in N supply for plant roots, the depolymerization and subsequent mineralization of N exceeded the N lost from the plant in the exudate pulse.

Taken together, the theoretical model supported both hypotheses; (1) simulated exudation stimulated microbial biomass, exo-enzyme activity, and microbial respiration, and (2) the effects of exudation were stronger when the exudates contained N as well as C. The exuded N allowed for the synthesis of additional microbial biomass and exo-enzymes, which have low C : N ratios and thus high N requirements.

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3.2 Field experiments

Microbial biomass responded consistently to exudation treatments across the two experiments (Fig. 4a). Treatment with water or C alone had no significant effect on microbial biomass relative to the disturbance controls (ANOVA, $p > 0.1$), while treatment with C and N exudate mimics significantly increased microbial biomass in both experiments (ANOVA, $p < 0.05$). This effect was consistent regardless of the method employed to measure microbial biomass (i.e., SIR vs. CFE, Fig. 4a). These experimental results agree qualitatively with decomposition model simulations designed to match the experimental methods; microbial biomass responded little when these exudate pulses contained C only, while microbial biomass increased when the exudates also contained N (Fig. 4c).

Microbial respiration measured at the end of each field experiment was significantly increased by the addition of C-only and C + N exudate mimics relative to either the disturbance or water controls (Fig. 4b, ANOVA, $p < 0.01$). These field results were consistent with the model, which predicted an increase in microbial respiration following an exudate pulse, with no effect of exudate C:N on the magnitude of the increase (Fig. 4d). Modeled respiration exhibited high-frequency fluctuations (grey lines in Fig. 4d), as exudate pulses stimulated respiration in the short-term. The running averages of microbial respiration were nearly identical across the model runs (black and red lines in Fig. 4d). The only exception was that there was a lag in the response of respiration in the C-only pulse run. Microbial biomass increased only marginally in this run (Fig. 4c), so initially the rate of DOC uptake (and resulting respiration) was lower in this run compared to the run with C and N additions. Over time, the pool of DOC increased, which increased microbial DOC uptake (and the resulting respiration) following Michaelis–Menten kinetics. Thus, over the long-term, modeled microbial respiration was increased by additions of C-only or C + N (Fig. 4d), which was consistent with the field experimental results (Fig. 4b).

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In general, the activity of soil exo-enzymes was affected by the addition of C and N together, while the water and C-only treatments had little effect (Table 2, Fig. 5). In response to the addition of C and N, there was a significant increase in the activity of hydrolytic enzymes that decompose relatively labile substrates and a significant decline in the activity of oxidative enzymes that decompose more recalcitrant substrates such as lignin (Fig. 5). Overall, the water and C-only exudate mimics did not significantly affect enzyme activities (Fig. 5). There were some exceptions on an enzyme-by-enzyme basis: C-only exudates increased NAG activity in the first experiment, while the water addition increased NAG and proteolytic enzyme activities in the second experiment (Table 2). The addition of water or C alone had no effect on the pool of extractable amino acids in the soil (ANOVA, $p > 0.1$), while the exudation of C and N together significantly increased the concentration of amino acids relative to the other treatments (ANOVA, $p < 0.05$, Table 2).

4 Discussion

The model and field experiments suggest that the microbial responses to exudate mimics were constrained by N supply. Exuding C alone had no effect on microbial activity other than to increase microbial respiration, whereas exuding C and N together stimulated microbial biomass, respiration, and the activity of exo-enzymes that depolymerize labile SOM. These results are consistent with the predictions derived from the second hypothesis, which stated that N availability constrains the microbial response to C exudates. The first hypothesis, which stated that exudates alone could stimulate microbial activity in rhizosphere soils, was conditionally supported. C-only exudate mimics were not sufficient to stimulate microbial and exo-enzyme activity in rhizosphere soils, while the combination of C and N exudation was sufficient to stimulate microbial biomass, microbial respiration, and the activity of exo-enzymes that decompose labile C sources (Figs. 4 and 5). By specifically isolating the effects of C vs. that of C and N, this study confirms the often assumed cause-and-effect relationship between root exudation and

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enhanced microbial activity in rhizosphere soils, but also suggests that exudate stoichiometry is an important driver of microbial activity in rhizosphere soils.

The model and field data suggest that rhizosphere effects are stronger when the exuded compounds meet microbial demands for multiple elements simultaneously. This conclusion is consistent with data from a variety of biomes where experimental additions of C- and N-containing substrates stimulated microbial activity in lab and field conditions (Vance and Chapin, 2001; Boberg et al., 2008; Sorensen et al., 2008; Brown et al., 2009; Currey et al., 2010; Krashevskaya et al., 2010; Lavoie et al., 2011; but see Yamasaki et al., 2011). Exudate effects on soil microbial function are also consistent with recent in situ observations of root and mycorrhizal effects in temperate forest soils (Phillips and Fahey, 2005, 2006; Turpault et al., 2007; Phillips et al., 2011). In temperate forests similar to those studied here, Brzostek and Finzi (2011) found that the presence of tree roots stimulated microbial and exo-enzyme activity relative to soils containing only ectomycorrhizal fungal hyphae. Thus, the delivery of exudate mimics alone, as studied here, appears to recreate the observed stimulation of microbial activity by roots in actual rhizosphere soils. This suggests that exudation may be sufficient to explain the observed acceleration of microbial activity and biogeochemical cycling in rhizosphere soils.

This study suggests that N-compounds in root exudates can affect rhizosphere processes by providing substrates for the synthesis of N-rich microbial biomass and exo-enzymes. However, it is not clear from the literature whether the exudation of N-containing compounds is a common and important mechanism across all plant-soil relationships. On one hand, it is well established that roots release amino acids to soils (Bowen, 1969; Rovira, 1969; Smith, 1976; Jaeger et al., 1999; Phillips et al., 2006), but solution-culture studies often find that the rate of root amino acid uptake is similar to or greater than efflux rates (Jones and Darrah, 1994b; Jones et al., 2009). Hence a logical conclusion is that roots re-absorb the amino acids they exude and that amino acids may not contribute to the stimulation of microbial activity in the rhizosphere (but see Phillips et al., 2004). On the other hand, in situ analysis of genetically-modified biosensors

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(reviewed by Cardon and Gage, 2006) demonstrate that bacteria acquire and use tryptophan in grass rhizospheres (Jaeger et al., 1999), as well as proline and lysine in corn rhizospheres (Vilchez et al., 2000; Espinosa-Urgel and Ramos, 2001), but not in bulk soils. This indicates that microbes acquire at least some of the amino acids exuded from roots into soils, possibly because physicochemical reactions in soils make amino acids subject to a broader array of fates relative to solution culture. In addition to exudation, root turnover and cell sloughing input a substantial quantity of N to soil, resulting in a “rhizodeposition” flux of N on the order of ~ 10 % of total plant N (reviewed by Jones et al., 2009). Thus, roots release N to soils in a variety of forms; this study suggests that this alleviates microbial N-limitation and, combined with C-exudates, stimulates microbial and exo-enzyme activity, which may prime the decomposition of SOM and release nutrients available for plant uptake.

Modeled microbial biomass was more responsive to N in exudates than was measured in the field experiments (Fig. 4a vs. c). This may have occurred because the model did not include the complexity of real soil communities, which are characterized by multiple functional groups of microorganisms, leading to feedbacks on microbial biomass and activity from higher trophic levels. Predators of rhizosphere microbes, particularly protozoa, can influence C and N cycling in the rhizosphere with concomitant effects on root-N availability (Griffiths, 1990; Zwart et al., 1994; Bonkowski, 2004; Cardon and Gage, 2006). Grazing of rhizosphere microbes may have constrained the response of microbial biomass to the addition of exudate mimics. We did not study these multi-trophic interactions, but this would be an interesting area for future research.

The responses of individual enzyme to exudate mimics were largely consistent from a stoichiometric perspective, although the results of the second experiment were more variable. In the first experiment, the release of exudate mimics containing only C stimulated the activity of an enzyme that decomposes N-containing substrates (NAG), while the exudate mimics containing C and N stimulated the activity of P releasing enzymes (AP) and increased soil N availability (extractable amino acids increased by 130 % relative to water or dry controls, Table 2). Thus, the first experiment suggested that C

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5 addition exacerbated microbial-N limitation, while C and N addition induced microbial-P limitation. The results of the second experiment were less clear. While AP and soil amino-acid pools responded similarly in both experiments, water addition alone had a significant effect in experiment 2, and increased NAG activity relative to all other treatments (Table 2). The same result was obtained when the samples were re-analyzed (*data not shown*), so this is not likely the result of a procedural error. It is possible that recovery from drought conditions preceding the time of soil sampling for the second experiment increased substrate supply across all treatments (Fig. B1), such that the exudate additions had a smaller relative effect on overall soil substrate availabilities. 10 Alternatively, the strong water effect may have simply been a sampling effect associated with the high spatial variability characteristic of soils. In general, the responses of individual enzymes across these experiments was stoichiometrically consistent and reflected inducible exo-enzyme production in response to nutrient supply and demand (Koch, 1985; Allison and Vitousek, 2005).

15 The differential response of enzymes targeting labile vs. recalcitrant SOM to treatment with exudate mimics (Fig. 5) suggests that the exudation of labile compounds from plant roots may increase the rate at which relatively labile SOM is decomposed, while reducing the decomposition rate of more recalcitrant SOM. That is, root exudation may prime the decomposition of relatively labile SOM by inducing exo-enzyme production with neutral or even negative effects on the priming of recalcitrant SOM. This result is generally consistent with the theory that soil microbes constitutively produce exo-enzymes at some low level that allows the microbes to detect environmental levels of insoluble polymers (e.g., cellulose) by the rate of monomer supply (e.g., glucose uptake into microbial cells); this allows for the inducible production of additional exo-enzymes targeting the insoluble polymer (Koch, 1985; Chróst, 1991; Koroljova-Skorobogatko et al., 1998; Shackle et al., 2000; Allison and Vitousek, 2005). Under this framework, root exudation of labile monomers (e.g., glucose) would largely stimulate the decomposition of labile SOM (e.g., cellulose). It is also possible that increased labile C supply from exudation leads to a change in microbial community composition, particularly 25

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favoring *r*-selected copiotrophic microbes with limited oxidative enzyme production at the expense of *K*-selected oligotrophic microbes that presumably have greater enzymatic diversity to decompose recalcitrant SOM (Fontaine et al., 2003; Fierer et al., 2007; Guenet et al., 2010). We have no data to address microbial community change in the current study, but labile C additions to temperate forest soils have been shown to increase the relative abundance of copiotrophic Bacteroidetes and β -Proteobacteria (Fierer et al., 2007). Finally, we speculate that root exudation primarily affects the decomposition of relatively labile SOM pools, but that plants are likely capable of stimulating the decomposition of recalcitrant SOM pools through other mechanisms such as C allocation to mycorrhizal fungal associates, which decompose a wide range of SOM substrates and transfer large quantities of nutrients to plant hosts (Chalot and Brun, 1998; Hodge et al., 2001; Hobbie and Hobbie, 2006; Talbot et al., 2008; Kaiser et al., 2011; Pritsch and Garbaye, 2011).

5 Conclusions

Analysis of a theoretical model of decomposition and two field experiments show that the simultaneous exudation of C and N elicits a larger microbial and enzymatic response in the rhizosphere than the exudation of C alone. This suggests that plants may be able to influence microbial activity and nutrient availability in the rhizosphere through the exudation of compounds that simultaneously meet microbial demands for multiple elements. Our analyses support the idea that exudation of C + N was sufficient to recreate known rhizosphere effects (e.g., higher rates of microbial biomass, respiration, and exo-enzyme activities), highlighting the importance of root exudation to belowground ecology. This study confirms a cause-and-effect relationship between root exudation and enhanced microbial activity in rhizosphere soils, and suggests that exudate stoichiometry is an important and underappreciated driver of microbial activity.

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Appendix A

A1 Model description

The model of decomposition used in this study was based on the published equations of Schimel and Weintraub (2003), modified using some equations from Allison et al. (2010). Here, we defined all of the model equations and parameters. Model parameters, pools, and fluxes were defined in Tables A1–A3.

We made four modifications to the model of Schimel and Weintraub (2003). (1) The original model assumed microbes instantaneously took up all available dissolved nutrients (i.e. DOC and DON). While this approach may be suitable for modeling steady-state conditions, it does not accurately reflect time-lags regarding the effect of substrate supply on microbial activity. Thus, we modeled microbial DOC and DON uptake using Michaelis–Menten kinetics as in Allison et al. (2010). (2) The equation governing inorganic-N immobilization (Eq. 18 in Schimel and Weintraub, 2003) was reversed in the original publication; microbes could immobilize N under C-limitation, but immobilization of N was not permitted when microbes were N-limited. We corrected this error by reversing the equation so that microbes could take up inorganic N when in a N-limited state. (3) The Schimel and Weintraub (2003) model did not include inorganic-N sinks, and as a consequence the pool of inorganic N grew without bound as the model was run forward. By allowing microbial-N immobilization under N-limited conditions (change 2, above) the lack of inorganic-N losses gave microbes access to a virtually unlimited supply of inorganic N, which is not realistic. Inorganic N is removed from soils relatively quickly (Berntson and Aber, 2000; Robertson and Groffman, 2007) through root-uptake (Perakis and Hedin, 2001), leaching (Vitousek and Melillo, 1979; Lovett et al., 2002), abiotic interactions with soil minerals (Davidson et al., 2003), denitrification (Potter et al., 1996; Seitzinger et al., 2006), NH₃ volatilization (Schlesinger and Peterjohn, 1991; Bouwman et al., 1997), as well as microbial uptake (Zak et al., 1990; Davidson et al., 1992). Combinations of these mechanisms operate at varying temporal

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scales in undisturbed forests (e.g., Perakis and Hedin, 2001). To avoid the complexity of adding these processes to the model, we modeled inorganic-N loss as a simple first-order kinetic process by assuming that 40 % of the inorganic-N pool was lost at each time-step. This modification reduced the equilibrium value for the inorganic-N pool from infinity to $\sim 3.5 \mu\text{g N g soil}^{-1}$ using the default parameter values (Table 1), which corresponds well with measurements of inorganic N in the temperate forest soils used for the field experiments ($3.7 \pm$ standard deviation of $1.6 \mu\text{g N g soil}^{-1}$, J. E. Drake, unpublished data; Brzostek et al., 2011). This change gave microbes access to a realistic pool of mineral N. (4) Schimel and Weintraub (2003) modeled enzyme production to be a constant fraction of microbial DOC uptake. When multiple exudate pulses were added to a model using this formulation, microbes went extinct (i.e., microbial biomass C and N became negative) because microbes were “forced” to produce large amounts of enzymes at the expense of their own biomass. To avoid this unrealistic scenario, we modeled the rate of enzyme production to be directly proportional to microbial biomass as in Allison et al. (2010).

A2 Decomposition of soil organic C and N (SOC and SON)

Insoluble soil organic C and N (SOC and SON) were assumed to be present in saturating amounts at the microbial scale as in Schimel and Weintraub (2003). The rate of depolymerization of SOC and SON into soluble forms of dissolved organic C and N (DOC and DON) was assumed to follow Michaelis–Menten dynamics related to the quantity of soil enzymes. SOC and SON depolymerization (D_c and D_n) were linked by the soil C : N ratio.

$$D_c = \kappa_D \times \frac{EC}{K_{es} + EC} \quad (\text{A1})$$

$$D_n = D_c \times CN_s \quad (\text{A2})$$

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A3 Microbial uptake of DOC and DON

As explained in the main text, we modified the approach of Schimel and Weintraub (2003) to model microbial uptake as in Allison et al. (2010). First, the maximum velocity (V_{\max}) and half-saturation constant (K_m) were calculated from soil temperature (T), which was set at a constant 20 °C for all of the modeling.

$$V_{\text{maxuptake}} = V_{\text{maxuptake0}} \cdot e^{-1 \cdot (E_{\text{uptake}} + \text{gasconst} \times (T + 273.15))} \quad (\text{A3})$$

$$K_{\text{muptake}} = K_{\text{muptakeslope}} \cdot T + K_{\text{muptake0}} \quad (\text{A4})$$

Next, the rates of DOC and DON uptake were calculated:

$$U_c = V_{\text{maxuptake}} \cdot BC \cdot \text{DOC} \div (K_{\text{muptake}} + \text{DOC}) \quad (\text{A5})$$

$$U_n = V_{\text{maxuptake}} \cdot BN \cdot \text{DON} \div (K_{\text{muptake}} + \text{DON}) \quad (\text{A6})$$

Rates of uptake were bounded by available substrate: i.e., U_c could not exceed DOC supply, and U_n could not exceed DON supply.

A4 Microbial physiology

In the model, microbes used organic C and N resources to synthesize enzymes, produce more biomass, and to meet respiration requirements. Some aspects of physiology depended on whether microbes were limited by C or N resources. At each time step, if the uptake of DOC was not sufficient to meet microbial demand, the microbes were considered C-limited. Conversely, if DOC uptake exceeded microbial demand, the microbes were considered N-limited.

$$\text{If: } U_c < R_m + \frac{EP_c}{SUE} + U_n - EP_n \cdot \frac{CN_m}{SUE}; \quad \text{then C-limitation} \quad (\text{A7})$$

$$\text{If: } U_c \geq R_m + \frac{EP_c}{SUE} + U_n - EP_n \cdot \frac{CN_m}{SUE}; \quad \text{then N-limitation} \quad (\text{A8})$$

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The rate of enzyme production was directly proportional to microbial biomass, and enzymes decayed in a first-order process, as in Allison et al. (2010).

$$EP_c = K_{ep} \cdot BC \quad (A9)$$

$$EL_c = K_l \cdot EC \quad (A10)$$

The N transferred during enzyme production and loss were linked to Eqs. (A8) and (A9) by the enzyme C : N ratio.

$$EP_n = EP_c \div CN_{enz} \quad (A11)$$

$$EL_n = EL_c \div CN_{enz} \quad (A12)$$

Microbes utilized organic C to support respiration processes, such as maintenance, enzyme production, growth, and overflow metabolism (when in a N-limited state).

$$R_m = K_m \cdot BC \quad (A13)$$

$$R_e = EP_c \cdot (1 - SUE) / SUE \quad (A14)$$

$$\text{If C-limited, } R_g = U_c - \frac{EP_c}{SUE} - R_m \cdot 1 - SUE \quad (A15)$$

$$\text{If N-limited, } R_g = U_n + J_n - EP_n \cdot CN_m \cdot 1 - SUE / SUE \quad (A16)$$

$$\text{If C-limited, } R_o = 0 \quad (A17)$$

$$\text{If N-limited, } R_o = U_c - R_m - \frac{EP_c}{SUE} - U_n + J_n - EP_n \cdot \frac{CN_m}{SUE} \quad (A18)$$

The rate of microbial uptake of inorganic N (immobilization) was zero under C-limitation. However, immobilization occurred during N-limitation:

$$\text{If N-limited, } J_n = U_c - R_m - \frac{EP_c}{SUE} \cdot \frac{SUE}{CN_m} - EP_n - U_n \quad (A19)$$

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Microbes mineralized N when limited by C resources, but N mineralization was zero under N-limitation.

$$\text{If C-limited, } M_n = U_n - EP_n - U_c - R_m - \frac{EP_c}{SUE} \cdot \frac{SUE}{CN_m} \quad (\text{A20})$$

5 A5 Recycling of dead microbial biomass and enzymes

As in Schimel and Weintraub (2003) and Allison et al. (2010), we assumed that a fixed proportion (K_r) of the C and N contained in dead microbial biomass was returned to soluble (DOC and DON) pools, with the remainder returned to insoluble pools (SOC and SON).

$$10 \quad CY_c = K_t \cdot K_r \cdot BC \quad (\text{A21})$$

$$CY_n = CY_c \div CN_m \quad (\text{A22})$$

$$H_c = K_t \cdot (1 - K_r) \cdot BC \quad (\text{A23})$$

$$H_n = H_c \div CN_m \quad (\text{A24})$$

15 A6 Changes in pool size

After calculating the fluxes above, the model calculated the pool sizes at the next time step (i.e. at time step $i + 1$).

$$BC(i + 1) = BC(i) + U_c - CY_c - EP_c - R_o - H_c - R_e - R_m - R_g \quad (\text{A25})$$

$$BN(i + 1) = BN(i) + U_n - CY_n - EP_n - M_n + J_n - H_n \quad (\text{A26})$$

$$20 \quad EC(i + 1) = EC(i) + EP_c - EL_c \quad (\text{A27})$$

$$EN(i + 1) = EN(i) + EP_n - EL_n \quad (\text{A28})$$

$$DOC(i + 1) = DOC(i) + D_c - U_c + CY_c \quad (\text{A29})$$

$$DON(i + 1) = DON(i) + D_n - U_n + CY_n \quad (\text{A30})$$

During an exudation event, the quantity Pulse_C was also added to the DOC pool, and $\text{Pulse}_C/\text{CN}_{\text{pulse}}$ was added to the DON pool.

As explained in the main text, we assumed that 40% of the mineral N pool was lost to the aggregate activity of many un-modeled fluxes (e.g. leaching, plant uptake, volatilization, and immobilization with soil minerals).

$$N_0(i+1) = 0.6 \cdot N_0(i) + M_n - J_n \quad (\text{A31})$$

Appendix B

B1 Environmental conditions during the field experiments

We measured soil temperature at 5 cm depth (type-T thermocouple) and soil moisture at 4.5 cm depth (time-domain reflectometry probes, model CS615, Campbell Scientific, Logan, Utah, USA) as described previously (Savage et al., 2009). The nearby environmental measurement site has measured whole-ecosystem exchange of CO_2 since 1991 (EMS, Urbanski et al., 2007); these data were downloaded from the Harvard Forest data archive (Munger and Wofsy, 1999) and used as a reference to time the exudation experiments during seasonal periods that typically have high rates of ecosystem C uptake.

We performed two 50-day exudate addition experiments during the summer of 2011. The first experiment was performed during early- to mid-summer, a period characterized by increasing soil temperatures, decreasing soil water content (Fig. B1a), and high rates of net ecosystem C uptake (Fig. B1b). The second experiment was performed in the mid- to late-summer. During this time, soil temperatures declined slightly, and soil moisture increased (Fig. B1a). Net ecosystem C uptake is typically positive during this time period, although rates of C uptake are generally lower than the early- to mid-summer (Fig. B1b).

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References

- Ainsworth, E. A. and Long, S. P.: What have we learned from 15 years of free-air CO₂ enrichment (FACE)? A meta-analytic review of the responses of photosynthesis, canopy, *New Phytol.*, 165, 351–371, 2005.
- Aitkenhead, J. A. and McDowell, W. H.: Soil C : N ratio as a predictor of annual riverine DOC flux at local and global scales, *Global Biogeochem. Cy.*, 14, 127–138, 2000.
- Allison, S. D. and Vitousek, P. M.: Responses of extracellular enzymes to simple and complex nutrient inputs, *Soil Biol. Biochem.*, 37, 937–944, 2005.
- Allison, S. D., Wallenstein, M. D., and Bradford, M. A.: Soil-carbon response to warming dependent on microbial physiology, *Nat. Geosci.*, 3, 336–340, 2010.
- Anderson, J. P. E. and Domsch, K. H.: Physiological method for quantitative measurement of microbial biomass in soils, *Soil Biol. Biochem.*, 10, 215–221, 1978.
- Basu, U., Godbold, D., and Taylor, G. J.: Aluminum resistance in *Triticum aestivum* associated with enhanced exudation of malate, *J. Plant Physiol.*, 144, 747–753, 1994.
- Batjes, N. H.: Total carbon and nitrogen in the soils of the world, *Eur. J. Soil Sci.*, 47, 151–163, 1996.
- Berntson, G. M. and Aber, J. D.: Fast nitrate immobilization in N saturated temperate forest soils, *Soil Biol. Biochem.*, 32, 151–156, 2000.

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- Bertin, C., Yang, X., and Weston, L. A.: The role of root exudates and allelochemicals in the rhizosphere, *Plant Soil*, 256, 67–83, 2003.
- Blagodatsky, S., Blagodatskaya, E., Yuyukina, T., and Kuzyakov, Y.: Model of apparent and real priming effects: linking microbial activity with soil organic matter decomposition, *Soil Biol. Biochem.*, 42, 1275–1283, 2010.
- Boberg, J., Finlay, R. D., Stenlid, J., Nasholm, T., and Lindahl, B. D.: Glucose and ammonium additions affect needle decomposition and carbon allocation by the litter degrading fungus *Mycena epipterygia*, *Soil Biol. Biochem.*, 40, 995–999, 2008.
- Bonkowski, M.: Protozoa and plant growth: the microbial loop in soil revisited, *New Phytol.*, 162, 617–631, 2004.
- Boose, E. and Gould, E.: Shaler Meteorological Station (1964–2002), Harvard Forest Data Archive: HF000, 1999.
- Boose, E.: Fisher Meteorological Station (since 2001), Harvard Forest Data Archive: HF001, 2001.
- Borken, W., Davidson, E. A., Savage, K., Gaudinski, J., and Trumbore, S. E.: Drying and wetting effects on carbon dioxide release from organic horizons, *Soil Sci. Soc. Am. J.*, 67, 1888–1896, 2003.
- Borken, W., Savage, K., Davidson, E. A., and Trumbore, S. E.: Effects of experimental drought on soil respiration and radiocarbon efflux from a temperate forest soil, *Glob. Change Biol.*, 12, 177–193, 2006.
- Bouwman, A. F., Lee, D. S., Asman, W. A. H., Dentener, F. J., VanderHoek, K. W., and Olivier, J. G. J.: A global high-resolution emission inventory for ammonia, *Global Biogeochem. Cy.*, 11, 561–587, 1997.
- Bowen, G. D.: Nutrient status effects on loss of amides and amino acids from pine roots, *Plant Soil*, 30, 139–142, 1969.
- Brant, J. B., Sulzman, E. W., and Myrold, D. D.: Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation, *Soil Biol. Biochem.*, 38, 2219–2232, 2006.
- Brown, A. L. P., Garland, J. L., and Day, F. P.: Physiological profiling of soil microbial communities in a Florida scrub-oak ecosystem: spatial distribution and nutrient limitations, *Microb. Ecol.*, 57, 14–24, 2009.
- Brzostek, E. R. and Finzi, A. C.: Substrate supply, fine roots, and temperature control proteolytic enzyme activity in temperate forest soils, *Ecology*, 92, 892–902, 2011.

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- Brzostek, E. R., Greco, A., Drake, J. E., and Finzi, A. C.: Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils, *Biogeochemistry*, in review, 2012.
- Cardon, Z. G. and Gage, D. J.: Resource exchange in the rhizosphere: molecular tools and the microbial perspective, *Annu. Rev. Ecol. Evol. S.*, 37, 459–488, 2006.
- Chalot, M. and Brun, A.: Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas, *Fems Microbiol. Rev.*, 22, 21–44, 1998.
- Chróst, R. J.: Environmental control of the synthesis and activity of aquatic microbial ectoenzymes, in: *Microbial Enzymes in Aquatic Environments*, edited by: Chróst, R. J., Springer-Verlag, New York, 29–59, 1991.
- Coleman, K., Jenkinson, D. S., Crocker, G. J., Grace, P. R., Klir, J., Korschens, M., Poulton, P. R., and Richter, D. D.: Simulating trends in soil organic carbon in long-term experiments using RothC-26.3, *Geoderma*, 81, 29–44, 1997.
- Currey, P. M., Johnson, D., Sheppard, L. J., Leith, I. D., Toberman, H., van der Wal, R., Dawson, L. A., and Artz, R. R. E.: Turnover of labile and recalcitrant soil carbon differ in response to nitrate and ammonium deposition in an ombrotrophic peatland, *Glob. Change Biol.*, 16, 2307–2321, 2010.
- Dakora, F. D. and Phillips, D. A.: Root exudates as mediators of mineral acquisition in low-nutrient environments, *Plant Soil*, 245, 35–47, 2002.
- Davidson, E. A., Hart, S. C., and Firestone, M. K.: Internal cycling of nitrate in soils of a mature coniferous forest, *Ecology*, 73, 1148–1156, 1992.
- Davidson, E. A., Belk, E., and Boone, R. D.: Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest, *Glob. Change Biol.*, 4, 217–227, 1998.
- Davidson, E. A., Chorover, J., and Dail, D. B.: A mechanism of abiotic immobilization of nitrate in forest ecosystems: the ferrous wheel hypothesis, *Glob. Change Biol.*, 9, 228–236, 2003.
- Davidson, E. A., Savage, K. E., Trumbore, S. E., and Borken, W.: Vertical partitioning of CO₂ production within a temperate forest soil, *Glob. Change Biol.*, 12, 944–956, 2006.
- De Nobili, M., Contin, M., Mondini, C., and Brookes, P. C.: Soil microbial biomass is triggered into activity by trace amounts of substrate, *Soil Biol. Biochem.*, 33, 1163–1170, 2001.
- Dilkes, N. B., Jones, D. L., and Farrar, J.: Temporal dynamics of carbon partitioning and rhizodeposition in wheat, *Plant Physiol.*, 134, 706–715, 2004.

**Microbial response
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- Drake, J. E., Gallet-Budynek, A., Hofmockel, K. S., Bernhardt, E. S., Billings, S. A., Jackson, R. B., Johnsen, K. S., Lichter, J., McCarthy, H. R., McCormack, M. L., Moore, D. J. P., Oren, R., Palmroth, S., Phillips, R. P., Pippen, J. S., Pritchard, S. G., Treseder, K. K., Schlesinger, W. H., DeLucia, E. H., and Finzi, A. C.: Increases in the flux of carbon belowground stimulate nitrogen uptake and sustain the long-term enhancement of forest productivity under elevated CO₂, *Ecol. Lett.*, 14, 349–357, 2011.
- Duncan, D. B.: Multiple range and multiple F-tests, *Biometrics*, 11, 1–42, 1955.
- Espinosa-Urgel, M. and Ramos, J. L.: Expression of a *Pseudomonas putida* aminotransferase involved in lysine catabolism is induced in the rhizosphere, *Appl. Environ. Microbiol.*, 67, 5219–5224, 2001.
- Farrar, J., Hawes, M., Jones, D., and Lindow, S.: How roots control the flux of carbon to the rhizosphere, *Ecology*, 84, 827–837, 2003.
- Fierer, N., Bradford, M. A., and Jackson, R. B.: Toward an ecological classification of soil bacteria, *Ecology*, 88, 1354–1364, 2007.
- Finzi, A., Sinsabaugh, R., Long, T., and Osgood, M.: Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest, *Ecosystems*, 9, 215–226, 2006.
- Fontaine, S., Mariotti, A., and Abbadie, L.: The priming effect of organic matter: a question of microbial competition?, *Soil Biol. Biochem.*, 35, 837–843, 2003.
- Fontaine, S., Bardoux, G., Abbadie, L., and Mariotti, A.: Carbon input to soil may decrease soil carbon content, *Ecol. Lett.*, 7, 314–320, 2004.
- Gallardo, A. and Schlesinger, W. H.: Estimating microbial biomass nitrogen using the fumigation incubation and fumigation extraction methods in a warm-temperate forest soil, *Soil Biol. Biochem.*, 22, 927–932, 1990.
- Grayston, S. J., Vaughan, D., and Jones, D.: Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability, *Appl. Soil Ecol.*, 5, 29–56, 1997.
- Griffiths, B. S.: A comparison of microbially-feeding nematodes and protozoa in the rhizosphere of different plants, *Biol. Fertil. Soils*, 9, 83–88, 1990.
- Guenet, B., Danger, M., Abbadie, L., and Lacroix, G.: Priming effect: bridging the gap between terrestrial and aquatic ecology, *Ecology*, 91, 2850–2861, 2010.

- Harden, T., Joergensen, R. G., Meyer, B., and Wolters, V.: Soil microbial biomass estimated by fumigation extraction and substrate-induced respiration in 2 pesticide-treated soils, *Soil Biol. Biochem.*, 25, 679–683, 1993.
- Hedges, L. V. and Olkin, I.: *Statistical Methods for Meta-Analysis*, Academic Press, Orlando, FL, 1985.
- Hobbie, J. E. and Hobbie, E. A.: N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra, *Ecology*, 87, 816–822, 2006.
- Hodge, A., Campbell, C. D., and Fitter, A. H.: An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material, *Nature*, 413, 297–299, 2001.
- Jaeger, C. H., Lindow, S. E., Miller, S., Clark, E., and Firestone, M. K.: Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan, *Appl. Environ. Microbiol.*, 65, 2685–2690, 1999.
- Jones, D. L. and Darrah, P. R.: Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere, *Plant Soil*, 166, 247–257, 1994a.
- Jones, D. L. and Darrah, P. R.: Amino-acid influx at the soil-root interface of *Zea mays* L and its implications in the rhizosphere, *Plant Soil*, 163, 1–12, 1994b.
- Jones, D. L., Prabowo, A. M., and Kochian, L. V.: Kinetics of malate transport and decomposition in acid soils and isolated bacterial populations: the effect of microorganisms on root exudation of malate under Al stress, *Plant Soil*, 182, 239–247, 1996.
- Jones, D. L.: Organic acids in the rhizosphere – a critical review, *Plant Soil*, 205, 25–44, 1998.
- Jones, D. L., Owen, A. G., and Farrar, J. F.: Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts, *Soil Biol. Biochem.*, 34, 1893–1902, 2002.
- Jones, D. L., Hodge, A., and Kuzyakov, Y.: Plant and mycorrhizal regulation of rhizodeposition, *New Phytol.*, 163, 459–480, 2004.
- Jones, D. L., Nguyen, C., and Finlay, R. D.: Carbon flow in the rhizosphere: carbon trading at the soil-root interface, *Plant Soil*, 321, 5–33, 2009.
- Kaiser, C., Fuchslueger, L., Koranda, M., Gorfer, M., Stange, C. F., Kitzler, B., Rasche, F., Strauss, J., Sessitsch, A., Zechmeister-Boltenstern, S., and Richter, A.: Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation, *Ecology*, 92, 1036–1051, 2011.

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- Koch, A. L.: The macroeconomics of bacterial growth, in: *Bacteria in their Natural Environments*, edited by: Fletcher, M. and Floodgate, G. D., Academic Press, London, 1–42, 1985.
- Koroljova-Skorobogatko, O. V., Stepanova, E. V., Gavrilova, V. P., Morozova, O. V., Lubimova, N. V., Dzchafarova, A. N., Jaropolov, A. I., and Makower, A.: Purification and characterization of the constitutive form of laccase from the basidiomycete *Coriolus hirsutus* and effect of inducers on laccase synthesis, *Biotechnol. Appl. Biochem.*, 28, 47–54, 1998.
- 5 Krashevskaya, V., Maraun, M., Ruess, L., and Scheu, S.: Carbon and nutrient limitation of soil microorganisms and microbial grazers in a tropical montane rain forest, *Oikos*, 119, 1020–1028, 2010.
- 10 Kuzyakov, Y., Hill, P. W., and Jones, D. L.: Root exudate components change litter decomposition in a simulated rhizosphere depending on temperature, *Plant Soil*, 290, 293–305, 2007.
- Langley, J. A., McKinley, D. C., Wolf, A. A., Hungate, B. A., Drake, B. G., and Megonigal, J. P.: Priming depletes soil carbon and releases nitrogen in a scrub-oak ecosystem exposed to elevated CO₂, *Soil Biol. Biochem.*, 41, 54–60, 2009.
- 15 Lavoie, M., Mack, M. C., and Schuur, E. A. G.: Effects of elevated nitrogen and temperature on carbon and nitrogen dynamics in Alaskan arctic and boreal soils, *J. Geophys. Res.-Biogeo.*, 116, G03013, doi:10.1029/2010jg001629, 2011.
- Lipson, D. A., Schmidt, S. K., and Monson, R. K.: Links between microbial population dynamics and nitrogen availability in an alpine ecosystem, *Ecology*, 80, 1623–1631, 1999.
- 20 Littell, R. C., Stroup, W. W., and Freund, R. J.: Analysis of variance for balanced data, in: *SAS for Linear Models*, SAS Institute Inc., Cary, North Carolina, 33–90, 2002.
- Lovett, G. M., Weathers, K. C., and Arthur, M. A.: Control of nitrogen loss from forested watersheds by soil carbon: nitrogen ratio and tree species composition, *Ecosystems*, 5, 712–718, 2002.
- 25 Munger, J. W. and Wofsy, S.: EMS – Canopy-Atmosphere Exchange of Carbon, Water, and Energy, Harvard Forest Data Archive: HF004, 1999.
- Norby, R. J., O’Neill, E. G., Hood, W. G., and Luxmoore, R. J.: Carbon allocation, root exudation and mycorrhizal colonization of *Pinus echinata* seedlings grown under CO₂ enrichment, *Tree Physiol.*, 3, 203–210, 1987.
- 30 Parton, W. J., Stewart, J. W. B., and Cole, C. V.: Dynamics of C, N, P, and S in grassland soils – a model, *Biogeochemistry*, 5, 109–131, 1988.
- Parton, W. J., Scurlock, J. M. O., Ojima, D. S., Gilmanov, T. G., Scholes, R. J., Schimel, D. S., Kirchner, T., Menaut, J. C., Seastedt, T., Moya, E. G., Kamnalrut, A., and Kinyamario, J. I.:

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- Observations and modeling of biomass and soil organic-matter dynamics for the grassland biome worldwide, *Global Biogeochem. Cy.*, 7, 785–809, 1993.
- Paterson, E.: Importance of rhizodeposition in the coupling of plant and microbial productivity, *Eur. J. Soil Sci.*, 54, 741–750, 2003.
- 5 Perakis, S. S. and Hedin, L. O.: Fluxes and fates of nitrogen in soil of an unpolluted old-growth temperate forest, Southern Chile, *Ecology*, 82, 2245–2260, 2001.
- Phillips, D. A., Fox, T. C., and Six, J.: Root exudation (net efflux of amino acids) may increase rhizodeposition under elevated CO₂, *Glob. Change Biol.*, 12, 561–567, 2006.
- Phillips, D. A., Fox, T. C., King, M. D., Bhuvaneshwari, T. V., and Teuber, L. R.: Microbial products trigger amino acid exudation from plant roots, *Plant Physiol.*, 136, 2887–2894, 2004.
- 10 Phillips, R. P. and Fahey, T. J.: Patterns of rhizosphere carbon flux in sugar maple (*Acer saccharum*) and yellow birch (*Betula allegheniensis*) saplings, *Glob. Change Biol.*, 11, 983–995, 2005.
- Phillips, R. P. and Fahey, T. J.: Tree species and mycorrhizal associations influence the magnitude of rhizosphere effects, *Ecology*, 87, 1302–1313, 2006.
- 15 Phillips, R. P., Bernhardt, E. S., and Schlesinger, W. H.: Elevated CO₂ increases root exudation from loblolly pine (*Pinus taeda*) seedlings as an N-mediated response, *Tree Physiol.*, 29, 1513–1523, 2009.
- Phillips, R. P., Finzi, A. C., and Bernhardt, E. S.: Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO₂ fumigation, *Ecol. Lett.*, 14, 187–194, 2011.
- 20 Potter, C. S., Matson, P. A., Vitousek, P. M., and Davidson, E. A.: Process modeling of controls on nitrogen trace gas emissions from soils worldwide, *J. Geophys. Res.-Atmos.*, 101, 1361–1377, 1996.
- Pritsch, K. and Garbaye, J.: Enzyme secretion by ECM fungi and exploitation of mineral nutrients from soil organic matter, *Ann. For. Sci.*, 68, 25–32, 2011.
- Richardson, A. D. and O’Keefe, J.: Phenological differences between understory and overstory: a case study using the long-term Harvard Forest records, in: *Phenology of Ecosystem Processes*, edited by: Noormets, A., Springer, New York, 2009.
- 25 Robertson, G. P. and Groffman, P. M.: Nitrogen transformation, in: *Soil Microbiology, Biochemistry, and Ecology*, edited by: Paul, E. A., Springer, New York, NY, USA, 341–364, 2007.
- Rosenberg, M. S., Adams, D. C., and Gurevitch, J.: *Metawin: Statistical Software for Meta-Analysis: Version 2.0*, Sinauer Associates Inc., Sunderland, MA, 2000.
- 30

- Rovira, A. D.: Plant root exudates, *Bot. Rev.*, 35, 35–56, 1969.
- Rustad, L. E., Campbell, J. L., Marion, G. M., Norby, R. J., Mitchell, M. J., Hartley, A. E., Cornelissen, J. H. C., Gurevitch, J., and Gcte, N.: A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming, *Oecologia*, 126, 543–562, 2001.
- Ryan, P. R., Delhaize, E., and Jones, D. L.: Function and mechanism of organic anion exudation from plant roots, *Annu. Rev. Plant Phys.*, 52, 527–560, 2001.
- Saiya-Cork, K. R., Sinsabaugh, R. L., and Zak, D. R.: The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil, *Soil Biol. Biochem.*, 34, 1309–1315, 2002.
- Savage, K., Davidson, E. A., Richardson, A. D., and Hollinger, D. Y.: Three scales of temporal resolution from automated soil respiration measurements, *Agr. Forest Meteorol.*, 149, 2012–2021, 2009.
- Savage, K. E. and Davidson, E. A.: Interannual variation of soil respiration in two New England forests, *Global Biogeochem. Cy.*, 15, 337–350, 2001.
- Schimel, J. P. and Weintraub, M. N.: The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model, *Soil Biol. Biochem.*, 35, 549–563, 2003.
- Schimel, J. P. and Bennett, J.: Nitrogen mineralization: challenges of a changing paradigm, *Ecology*, 85, 591–602, 2004.
- Schlesinger, W. H. and Peterjohn, W. T.: Processes controlling ammonia volatilization from Chihuahuan desert soils, *Soil Biol. Biochem.*, 23, 637–642, 1991.
- Seitzinger, S., Harrison, J. A., Bohlke, J. K., Bouwman, A. F., Lowrance, R., Peterson, B., Tobias, C., and Van Drecht, G.: Denitrification across landscapes and waterscapes: a synthesis, *Ecol. Appl.*, 16, 2064–2090, 2006.
- Shackle, V. J., Freeman, C., and Reynolds, B.: Carbon supply and the regulation of enzyme activity in constructed wetlands, *Soil Biol. Biochem.*, 32, 1935–1940, 2000.
- Shi, S. J., Richardson, A. E., O’Callaghan, M., DeAngelis, K. M., Jones, E. E., Stewart, A., Firestone, M. K., and Condon, L. M.: Effects of selected root exudate components on soil bacterial communities, *FEMS Microbiol. Ecol.*, 77, 600–610, 2011.
- Smith, W. H.: Character and significance of forest tree root exudates, *Ecology*, 57, 324–331, 1976.
- Somers, E., Vanderleyden, J., and Srinivasan, M.: Rhizosphere bacterial signalling: a love parade beneath our feet, *Crit. Rev. Microbiol.*, 30, 205–240, 2004.

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Sorensen, P. L., Michelsen, A., and Jonasson, S.: Ecosystem partitioning of ¹⁵N-glycine after long-term climate and nutrient manipulations, plant clipping and addition of labile carbon in a subarctic heath tundra, *Soil Biol. Biochem.*, 40, 2344–2350, 2008.

5 Sterner, R. W., and Elser, J. J.: Biological chemistry: building cells from elements, in: *Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere*, Princeton University Press, Princeton, NJ, 44–79, 2002.

Talbot, J. M., Allison, S. D., and Treseder, K. K.: Decomposers in disguise: mycorrhizal fungi as regulators of soil C dynamics in ecosystems under global change, *Funct. Ecol.*, 22, 955–963, 2008.

10 Turpault, M. P., Gobran, G. R., and Bonnaud, P.: Temporal variations of rhizosphere and bulk soil chemistry in a Douglas fir stand, *Geoderma*, 137, 490–496, 2007.

Urbanski, S., Barford, C., Wofsy, S., Kucharik, C., Pyle, E., Budney, J., McKain, K., Fitzjarrald, D., Czikowsky, M., and Munger, J. W.: Factors controlling CO₂ exchange on timescales from hourly to decadal at Harvard Forest, *J. Geophys. Res.-Biogeo.*, 112, G02020, doi:10.1029/2006jg000293, 2007.

15 Vance, E. D. and Chapin, F. S.: Substrate limitations to microbial activity in taiga forest floors, *Soil Biol. Biochem.*, 33, 173–188, 2001.

Vilchez, S., Molina, L., Ramos, C., and Ramos, J. L.: Proline catabolism by *Pseudomonas putida*: cloning, characterization, and expression of the put genes in the presence of root exudates, *J. Bacteriol.*, 182, 91–99, 2000.

20 Vitousek, P. M. and Melillo, J. M.: Nitrate losses from disturbed forests: patterns and mechanisms, *Forest Sci.*, 25, 605–619, 1979.

Watanabe, K. and Hayano, K.: Seasonal-variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus spp.* in paddy field soil, *Soil Biol. Biochem.*, 27, 197–203, 1995.

25 Yamasaki, A., Tateno, R., and Shibata, H.: Effects of carbon and nitrogen amendment on soil carbon and nitrogen mineralization in volcanic immature soil in Southern Kyushu, Japan, *J. For. Res.-Jpn.*, 16, 414–423, 2011.

30 Zak, D. R., Groffman, P. M., Pregitzer, K. S., Christensen, S., and Tiedje, J. M.: The vernal dam – plant microbe competition for nitrogen in northern hardwood forests, *Ecology*, 71, 651–656, 1990.

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Zimmermann, M., Leifeld, J., Schmidt, M. W. I., Smith, P., and Fuhrer, J.: Measured soil organic matter fractions can be related to pools in the RothC model, *Eur. J. Soil Sci.*, 58, 658–667, 2007.

5 Zwart, K. B., Kuikman, P. J., and van Veen, J. A.: *Rhizosphere protozoa*: their significance in nutrient dynamics, in: *Soil Protozoa*, edited by: Darbyshire, J. F., CAB International, Wallingford, UK, 93–122, 1994.

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Table 1. Parameter sensitivity analysis of modeled net N mineralization and SOM decomposition following an exudate pulse.

Parameter	Description	Low value	Moderate value	High value	Sensitivity of net N mineralization	Sensitivity of decomposition of SOM
K_f	Decay rate for exo-enzymes	0.025	0.05	0.1	1.35	1.35
Exudate C : N	C : N ratio of exudate pulse	25	50	100	1.00	0.50
Exudate pulse size	Quantity of exudates delivered (μgC)	50	100	200	0.99	0.57
SUE	Substrate-use-efficiency	0.25	0.5	1	0.77	1.77
K_r	Proportion of microbial biomass recycled into DOM pools	0.43	0.85	1	0.70	0.20
K_{ep}	Proportionality of exo-enzyme production and microbial biomass	0.00025	0.0005	0.001	0.57	1.31
Soil C : N	C : N ratio of SOM	10	20	30	0.42	0.11
K_m	Microbial maintenance rate	0.005	0.01	0.02	0.31	0.43
K_t	Decay rate for microbes	0.006	0.012	0.024	0.27	0.56
Exo-enzyme C : N	C : N ratio of exo-enzymes	2	3	4	0.24	0.03
Microbe C : N	C : N of microbial biomass	3.5	7	14	0.15	0.58

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Table 2. Measured activity of soil exo-enzymes and availability of soil amino acids in response to exudate mimic delivery to intact forest soils in the field. Values reflect the mean (\pm SE) of 10 replicates. T indicates a significant effect of exudation treatment, E indicates a significant difference between the two experiments, and T \times E indicates a significant interaction. Within an experiment, treatments that share a letter are not significantly different.

Experiment	Treatment	Enzymes that decompose labile substrates					Enzymes that decompose recalcitrant substrates	
		AP nmol g ⁻¹ h ⁻¹	NAG nmol g ⁻¹ h ⁻¹	BG nmol g ⁻¹ h ⁻¹	Proteolytic μ g Ng ⁻¹ 4h ⁻¹	Amino acid pool size μ g Ng ⁻¹	PhenO _x nmol g ⁻¹ h ⁻¹	PerO _x nmol g ⁻¹ h ⁻¹
1	Control	291 (20) a	19.3 (3.4) a	123 (8) a	8.9 (0.7) ab	4.6 (0.5) a	696 (57) a	473 (62) a
1	Water	250 (23) a	17.6 (3.2) a	114 (14) a	6.8 (1.4) b	4.9 (0.4) a	698 (31) a	697 (47) b
1	C	258 (10) a	41.7 (3.4) b	122 (13) a	9.2 (1.3) ab	5.8 (0.7) a	580 (57) a	423 (35) ac
1	C+N	530 (57) b	54.2 (6.7) b	159 (14) b	12.9 (1.9) a	11.5 (1) b	399 (73) b	302 (54) c
2	Control	292 (20) a	11.1 (1.6) a	102 (16) a	5.3 (0.7) a	8.4 (0.6) a	620 (67) a	909 (146) a
2	Water	299 (32) a	52.9 (7.3) b	103 (8) a	9.3 (1.1) b	7.1 (0.9) a	470 (75) a	575 (102) b
2	C	348 (31) a	20.5 (2.6) a	116 (11) a	7.3 (0.6) ab	8.1 (0.5) a	648 (60) a	1050 (75) a
2	C+N	523 (37) b	15.7 (2.7) a	124 (12) a	7.5 (0.8) ab	10.2 (0.6) b	467 (58) a	841 (75) ab
Significant effects		T	T, E, T \times E	T, E	T, E, T \times E	T, E	T	E, T \times E

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Table A1. Model parameters.

List of parameter abbreviations, typical values, and definitions.		
K_{ep}	0.0005	Rate of enzyme production per unit microbial biomass (unit-less)
K_j	0.05	Enzyme decay constant (unit-less)
K_m	0.01	Microbial maintenance rate, includes cost of maintaining living cells and biomass turnover (unit-less)
SUE	0.5	Substrate use efficiency: the fraction of DOC uptake that is converted to biomass or enzymes (unit-less)
K_t	0.012	Proportion of microbial biomass that dies each time-step (unit-less)
K_r	0.85	Proportion of dead microbial biomass that is recycled to DOC and DON pools (unit-less). The remainder re-enters SOC and SON pools.
Pulse_C	50	Size of exudate pulse (μgCg^{-1})
CN_s	20	C : N ratio of the soil (SOC/SON)
CN_m	7	C : N ratio of the microbes
CN_{pulse}	100	C : N ratio of the exudate pulse
CN_{enz}	3	C : N ratio of the enzymes
$V_{\text{maxuptake0}}$	1.5×10^8	Pre-exponential rate of DOC uptake ($\mu\text{gDOCg}^{-1}\text{h}^{-1}$)
E_{uptake}	47	Activation energy of DOC uptake ($\text{kJ mol}^{-1}\text{C}^{-1}$)
Gasconst	0.008314	Universal gas constant ($\text{kJ mol}^{-1}\text{K}^{-1}$)
$K_{\text{muptakeslope}}$	0.015	Rate by which the K_m of DOC uptake increases with each increase in temperature ($\mu\text{gDOCg}^{-1}\text{C}^{-1}$)
K_{muptake0}	0.154	K_m of DOC uptake at 0°C (μgDOCg^{-1})
K_{es}	0.3	Michaelis–Menten half-saturation constant; enzyme pool size value at which the decomposition of SOC/SON is half-saturated.
kappa_D	1.0	Decomposition constant for SOC/SON pool.

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Table A2. Model pools.

BC	Microbial biomass C
BN	Microbial biomass N
EC	Enzyme C
EN	Enzyme N
N0	Mineral N
DOC	Dissolved organic C
DON	Dissolved organic N

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**Table A3.** Model fluxes.

D_c	Rate of conversion from SOC to DOC (decomposition)
D_n	Rate of conversion from SON to DON (decomposition)
U_c	Rate of microbial DOC uptake
U_n	Rate of microbial DON uptake
EP_c	Rate of enzyme C production
EP_n	Rate of enzyme N production
EL_c	Rate of enzyme C decay
EL_n	Rate of enzyme N decay
R_m	Maintenance respiration rate
R_e	Respiration to support the production of enzymes
R_g	Respiration to support growth of microbial biomass
R_o	Overflow respiration, when DOC is taken up in excess of microbial demand
J_n	Microbial immobilization of inorganic N
M_n	Rate of microbial N mineralization
CY_c	Recycling of dead microbial C back to DOC
H_c	Loss of dead microbial C to SOC
CY_n	Recycling of dead microbial N back to DON
H_n	Loss of dead microbial N to SON

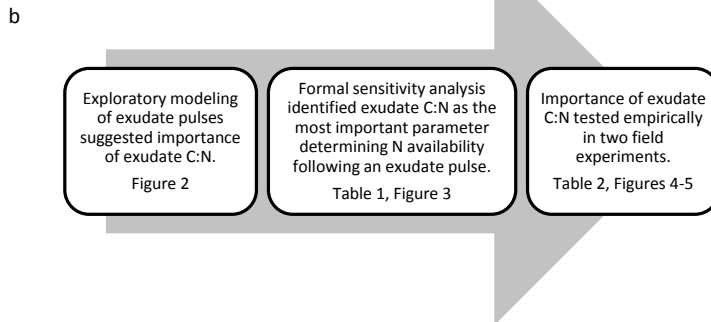
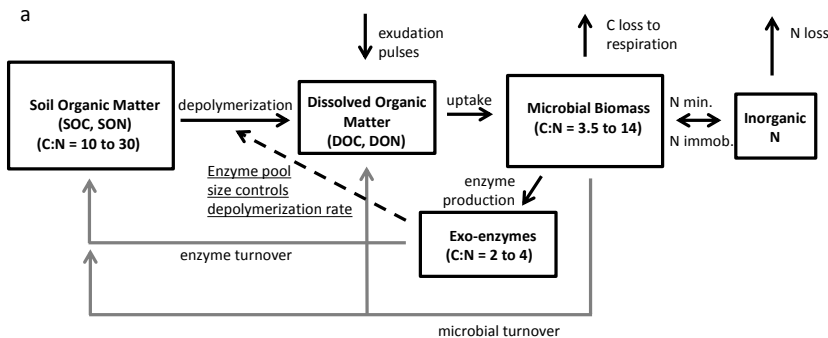


Fig. 1. Structure of the decomposition model **(a)** and graphical description of how the modeling motivated the field experiment **(b)**. In **(a)**, solid arrows refer to fluxes and boxes refer to pools. The turnover of dead microbes and inactivated enzymes into soil organic matter and dissolved organic matter pools are shown as grey arrows, and the importance of enzymes in depolymerizing soil organic matter into dissolved pools is shown with a dotted arrow. “N min.” refers to N mineralization, and “N immob.” refers to the immobilization of inorganic N into microbial biomass. A detailed description of the model equations can be found in Appendix A.

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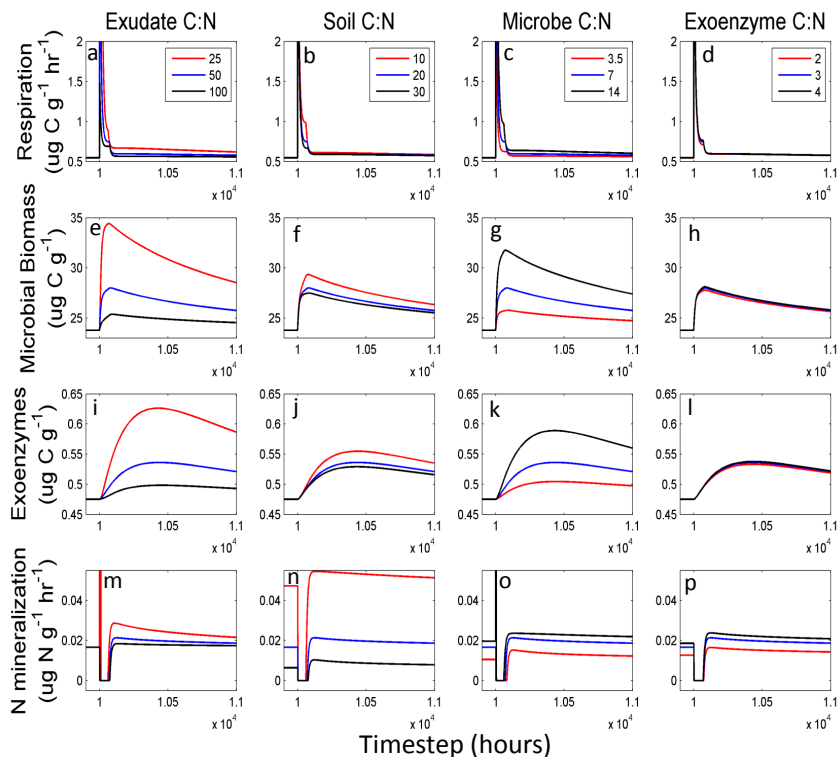


Fig. 2. Model responses of respiration, microbial biomass, soil enzymes, and N mineralization to an exudate pulse depend on stoichiometry. The model was “spun-up” for 1×10^4 timesteps to reach steady state and a pulse of DOC and DON was added. The model was run at the moderate value for each parameter (Table 1) except the parameter that was varied. In general, the response to an exudate pulse was greatest when N availability was high (low exudate C : N and low soil C : N) and microbial N demand was low (high microbial C : N and high enzyme C : N).

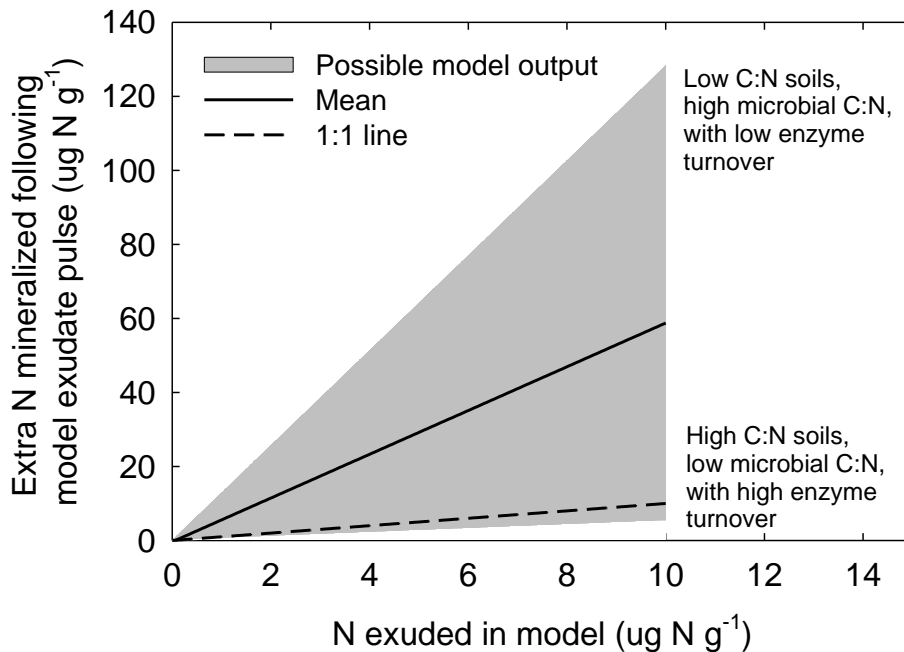


Fig. 3. Model results of the additional N mineralized following exudate pulses of varying N content. The cumulative extra N mineralized following an exudate pulse increased with increasing N exudation (solid line, $y = 5.9x - 0.4$) and was generally above the 1 : 1 line (dashed line). N exudation increased exo-enzyme activity which released N from SON. The grey area indicates possible model output, depending on parameter values.

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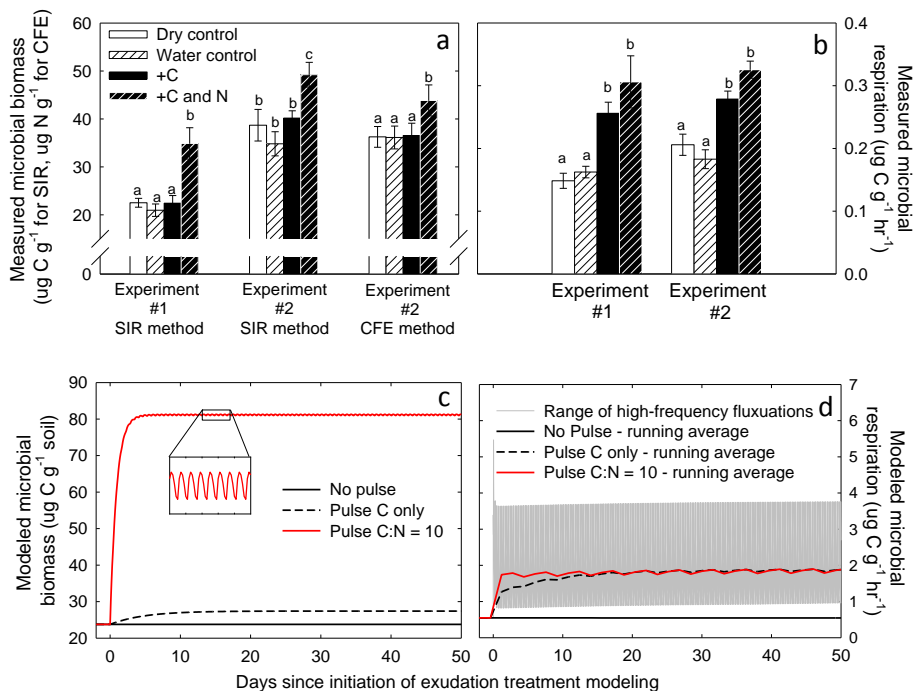


Fig. 4. The response of microbial biomass and respiration to exudation treatments in two field experiments and a theoretical model. Microbial biomass and respiration in the field experiments (a and b) were measured after 50 days of treatment, and 50-day model runs were produced for comparison (c and d). Both field experiments consisted of a dry disturbance control, a water control, and treatments with C-only and C and N exudate mimics ($n = 10$). Model simulations match the experimental treatments, but the water control was excluded. Treatments that do not share a letter were significantly different ($p < 0.05$). High-frequency fluctuations in the model simulations related to exudation events are shown with an inset (c) and grey lines (d).

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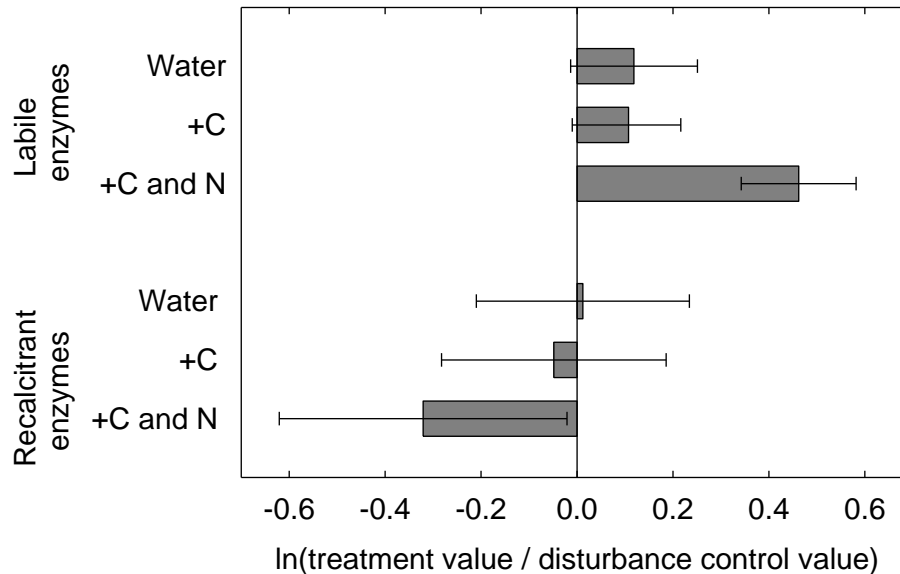


Fig. 5. Meta-analytic summary of the response of soil exo-enzyme activity to the delivery of exudate mimics across two field experiments. Experimental treatments included disturbance controls, water controls, C-only exudate mimics, and C + N exudate mimics. The response ratio of experimental treatments relative to disturbance controls is shown. Error bars reflect bias-corrected bootstrapped 95 % confidence intervals. Exo-enzymes were grouped into categories of enzymes that catalyze the decomposition of labile substrates (i.e. proteolytic, AP, NAG, and BG enzymes) and enzymes that catalyze the decomposition of recalcitrant substrates (e.g., the lignolytic enzymes PhenO_x and PerO_x).

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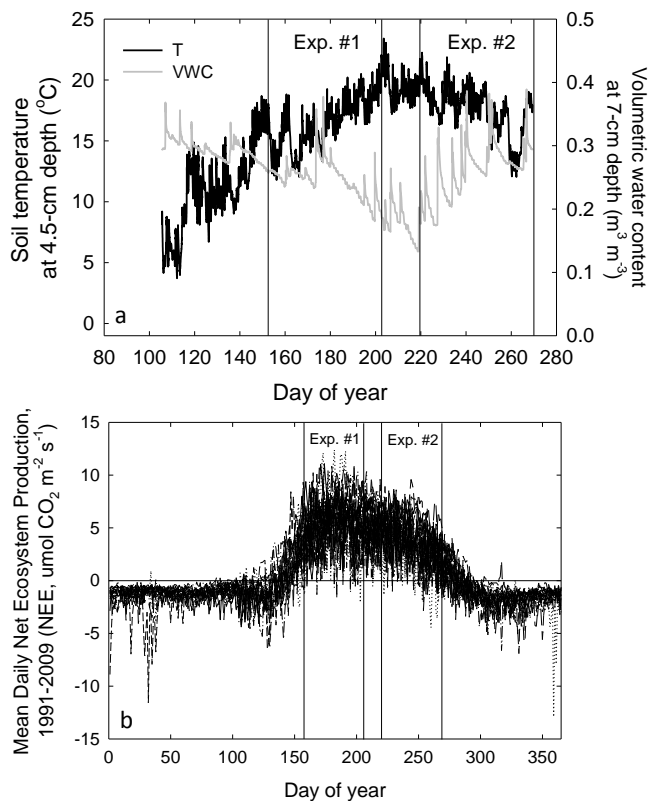



Fig. B1. Environmental attributes during field exudation treatments at Harvard Forest, MA, 2011 (a). There were two experiments (Exp. #1 and Exp. #2), demarcated by thin vertical black lines. Both experiments lasted 50 d. Net Ecosystem Production (NEP) of CO_2 measured at the Environmental Measurements Site (EMS) over the period 1991–2009 showed a consistent seasonal cycle (b).