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Resource stoichiometry mediates soil C loss and nutrient transformations in forest soils

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ABSTRACT

Root exudation is increasingly being recognized as an important driver of ecosystem processes; however, few studies have examined the degree to which variations in exudate stoichiometry and soil resources affect microbial controls of nutrient availability and decomposition. We added root exudate mimics of varying chemical quality to soils collected from two adjacent forest stands (one a ~70 year-old spruce plantation, the other a ~200 year-old spruce-fir forest) that differ strongly in N availability. The exudate treatments were added for 50 consecutive days, and included water (control), C alone, N alone, and three combinations of C and N that varied stoichiometrically (i.e., C:N ratio of 10, 50 and 100). Exudate additions containing little or no N promoted the greatest losses of soil C in two soils, with the greatest losses occurring in the moderately labile (i.e., acid-extractable) fraction of the low N plantation soils. However, despite the uniformity of priming effects between sites (~7% loss of soil C for both), there was little congruence in exudate-induced effects on microbial biomass and activity. In the plantation soils, exudates generally increased microbial biomass (especially fungi), accelerated N cycling and increased lignin-degrading enzyme activities relative to controls. In contrast, exudate additions to spruce-fir soils mostly decreased microbial biomass, decelerated N cycling, and had variable impacts on lignin-degrading enzyme activities (decreased phenol oxidase but increased peroxidase). Collectively, this study suggests that while root exudates with low C and N have the potential to accelerate soil C losses by stimulating microbes to mine N from soil organic matter, the consequences of exudate inputs on nutrient fluxes are less predictable, and may hinge on the recalcitrance of (soil organic matter) SOM, N availability and microbial communities.

1. Introduction

The transfer of carbon (C) from tree roots to the surrounding soil (i.e., the rhizosphere) is increasingly being recognized as a critical driver of ecosystem processes (Hogberg et al., 2001; Cheng and Kuzyakov, 2005; Finzi et al., 2015), ecosystem sensitivity to global change (Phillips et al., 2011), and biospheric feedbacks to climate (Bardgett et al., 2008; Heimann and Reichstein, 2008). Plant roots release between 5 and 20% of total photosynthate C as root exudates, mostly as soluble sugars, amino acids, or organic acids (Grayston et al., 1996). By exuding compounds of varying chemical quality, roots regulate microbial physiology and extracellular enzyme production in ways that can both accelerate or decelerate SOM decomposition and increase or decrease nutrient availability (Cheng et al., 2014). Consequently, there is a need to develop a theoretical and predictive understanding of exudate impacts on soil biogeochemistry, in order to develop improved strategies that maximize ecosystem services while minimizing environmental degradation.

In forests, most studies of the biogeochemical impacts of exudates have focused on quantifying exudation rates or rhizosphere C fluxes (Phillips and Fahey, 2005; Phillips et al., 2011; Brzostek et al., 2013; Yin et al., 2013; Cheng et al., 2014). Much less is known about variation in the chemical quality of exudates (among tree species or within the same species growing in

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different environmental conditions), and how such variation can influence microbial communities and ecosystem processes. Most exudates are organic compounds (Smith, 1976; Grayston et al., 1996) that have C:N ratios greater than those of the microbial biomass (Cleveland and Liptzin, 2007). This can create strong competition between roots and microbes for N in the rhizosphere, leading to N immobilization and exacerbated nutrient limitation for plants (Jackson et al., 2008; Frank and Groffman, 2009). Moreover, tree species can differ in their exudate stoichiometry (Smith, 1976), and such differences have the potential to alter bacterial (Eilers et al., 2010), fungal (Broeckling et al., 2008) or both bacteria and fungal (de Graaff et al., 2010) communities, and consequently SOM decomposition and nutrient cycling via priming effects (Landi et al., 2006; Drake et al., 2013).

Soil properties, such as the capacity to mineralize N, can also affect how exudate inputs affect SOM decomposition (Dijkstra et al., 2013; Murphy et al., 2015). Priming effects (PE) occur when inputs of labile C (or N) accelerate or decelerate microbial decomposition of SOM (positive or negative PE, respectively) (Kuzyakov and Domanski, 2000). To date, the effects of N availability of soil substrates on SOM decomposition have been conflicting, with reports of positive (de Graaff et al., 2006; Fontaine et al., 2011), negative (Janssens et al., 2010; Blagodatskaya et al., 2007) or no (Liljeroth et al., 1994) effects. Numerous competing hypotheses have been proposed to explain these variable impacts. The "stoichiometric decomposition theory" predicts that microbial activity is highest and SOM decomposition rates are maximal when the C:N ratio of microbial substrates matches the cellular and physiological demands of microbes (Hessen et al., 2004). Under this scenario, priming effects are predicted to be greatest in soils with high inorganic N (i.e., narrow C:N). In contrast, the "microbial nitrogen mining hypothesis" predicts that priming should be greatest in low N soils (i.e., wide C:N) given that microbes will use labile C as an energy source to decompose SOM to access N (Moorhead and Sinsabaugh, 2006). Similarly, the "preferential substrate utilization" hypothesis predicts that priming should be lowest in high N soils (i.e., narrow C:N), under the assumption that these soils contain an abundant supply of C (from exudates) and inorganic N (from soils). Consequently, microbes have little "need" to decompose SOM. However, because some N is needed by microbes to synthesize proteins such as extracellular enzymes, this hypothesis predicts that priming effects should be greatest in soils with low-moderate levels of inorganic N (Drake et al., 2013; Finzi et al., 2015). While these hypotheses all have strong theoretical support, few experiments have been conducted to directly test how variation in exudate stoichiometry interacts with soil N availability to regulate priming effects and nutrient fluxes (Yin et al., 2014).

In the present study, we investigated the impacts of root exudate stoichiometry on microbial processes and soil C dynamics in two adjacent forests: a ~70 year-old dragon spruce (*Picea asperata*) plantation (hereafter spruce plantation) characterized by low soil N, and a ~200 year-old forest dominated by spruce (*P. asperata*) and fir (*Abies faxoniana*) (hereafter spruce-fir) characterized by high soil N. Because the forests have underlying soils, differences in soil N availability between the two forests provided a unique opportunity to examine whether N availability influences the impacts of exudate additions on SOM decomposition. The aim of this study was to investigate the degree to which priming effects depend on substrate stoichiometry, N availability, and microbial communities, by adding exudate mimics to field soils. We hypothesized that losses of soil organic C owing to exudate addition (i.e., priming effects) would be greatest in spruce plantation soils with the lowest soil N (e.g., plantation soils receiving C only) and least in spruce-fir soils with the highest soil N (e.g., spruce-fir soils receiving N).

Table 1
Soil physical, chemical and biological properties for the spruce plantation and spruce-fir soils (means of three replicates ± SD).

Properties	Plantation soil	Spruce-fir soil
pH (H_2O)	6.73 ± 0.03	6.97 ± 0.02
Soil moisture (%)	35.36 ± 1.64	37.92 ± 2.66
Bulk density ($g\,cm^{-3}$)	1.23 ± 0.05	1.06 ± 0.07
Total C ($mg\,g^{-1}$)	49.22 ± 1.18	114.87 ± 1.10
Total N ($mg\,g^{-1}$)	3.20 ± 0.12	8.46 ± 0.31
C-to-N ratio	15.41 ± 0.55	13.59 ± 0.51
Gross nitrification rate ($\mu g\,N\,kg^{-1}\,h^{-1}$)	138.54 ± 8.96	178.95 ± 7.42
Net N mineralization rate ($mg\,kg^{-1}\,day^{-1}$)	2.95 ± 0.45	20.08 ± 2.36

2. Materials and methods

2.1. Sites and soil preparation

Soils were sampled to 20 cm depth from two adjacent forests in October of 2013. One forest is a dragon spruce plantation (c.a. 70 years old) while the other is an old-growth spruce-fir forest (c.a. 200 years old). Both study sites are located at the Miyaluo Experimental Forest of Lixian County, Sichuan province (31°35′N; 102°35′E; 3150 m a.s.l.). The mean annual temperature is 8.9 °C, with a maximum monthly mean air temperature of 12.6 °C in July and a minimum of −8 °C in January. Annual precipitation ranges from 600 to 1100 mm. Soils at both sites are typical brown forests soils and are classified as a Cambic Umbrisols according to IUSS Working Group (2007). At each site, twelve soil samples were randomly taken from the topsoil (0–20 cm) with a 5 cm diameter stainless steel core and then bulked together. Fresh soils were sieved (2 mm) at field moisture content and stored at 4 °Cprior to use. Each composite soil sample was divided into two subsamples. One subsample was stored for measuring soil physical, chemical and biological properties (see Table 1). Specially, gross nitrification rate was measured using a Barometric Process Separation (BaPS) instrument (UMS GmbH Inc., Munich, Germany) through laboratory incubations, as described by Sun et al. (2009). The second subsample was processed for exudate addition experiment.

2.2. Root exudate mimics

The preliminary experiments indicated that the C:N ratios of root exudate for *P. asperata* species under field conditions generally ranged from 10 to 20. Thus, five "root exudate mimics" were made by mixing compounds commonly reported as constituents of root exudates in the present study (Smith, 1976; Grayston et al., 1996; Drake et al., 2013; Keiluweit et al., 2015). The five treatments included: C-only, N-only, and combinations of C and three N levels (C:N ratio of 10, 50 and 100). The exudate mimics containing both C and N were comprised of a sugar (glucose), an organic acid (citric acid), and an amino acid (glutamic acid), while the mimics containing C only included only glucose and citric acid (Table 2). The concentration of C added to the soils was chosen to mimic annual exudate C flux for the 70-year-old spruce plantation into top 15 cm soils: calculated $6\,mg\,kg^{-1}$ dry soil per day whereas N concentrations varied with corresponding C:N ratio for all the treatments. For the incubation, 100 g fresh soil was used for each treatment. The preliminary experiment showed that soil water losses through evaporation were largely counterbalanced by the addition of 10 mL solution/deionized water per day during the experimental period. Thus, each specimen was treated with 10 mL of $60\,mg\,C\,L^{-1}$root exudate solution once a day in order to maintain soil water content at original soil moisture and mimic exudate C input (i.e., 0.6 mg exudate C per 100 g soil). The controls were

Table 2
Chemical components and contents of simulated root exudates with different C:N stoichiometry.

Treatment	Glucose (g)	Citric acid (g)	Glutamic acid (g)	NH_4Cl (g)	Deionized water (ml)
Control(CK)	–	–	–	–	1000
N-only	–	–	–	0.0229	1000
C:N = 10	0.0753	0.0753	0.0168	0.0168	1000
C:N = 50	0.0830	0.0830	0.0034	0.0034	1000
C:N = 100	0.0840	0.0840	0.0017	0.0017	1000
C-only	0.0850	0.0850	–	–	1000

treated in the same way using deionized water. All the exudate solutions were re-prepared weekly to minimize the effects of microbial contamination.

First, 100 g fresh soil at ambient field moisture content was placed in specimen cup (4 cm diameter, 7 cm high). Before exudate additions, soils were pre-incubated for 10 days at 25 °Cto stabilize the disturbance of previous soil preparation and to recover microbial activity that may have been diminished during the storage period. Following this stabilization period, each treatment was applied by syringe in 10 mL exudate mimics solution once a day. After exudate addition, soil samples were carefully mixed with a glass rod to distribute the material homogeneously, and placed in a constant temperature incubator (25 °C) for 50 days. Each treatment had three replicates.

2.3. Soil nutrients, microbial biomass and enzymes assay

Total C and N in all samples were analyzed using an elemental analyzer (vario MACRO cube, Elementar, Germany). A two-step acid hydrolysis procedure with H_2SO_4 as the extractant was used to determine labile and recalcitrant C (Rovira and Vallejo, 2002). Briefly, 20 mL 2.5 M H_2SO_4 was added to 500 mg soil, and the sample was hydrolyzed for 30 min at 105 °C insealed Pyrex tubes, after which the hydrolysate was recovered by centrifugation and decantation. The residue was washed with 20 mL de-ionized water and the washing added to the hydrolysate. This hydrolysate was taken as labile pool I (LPI) and analyzed for labile pool I carbon (LPI-C). The remaining residue was hydrolyzed with 2 mL 13MH_2SO_4 overnight at room temperature under continuous shaking. The concentration of the acid was then brought down to 1 M by dilution with de-ionized water and the sample was hydrolyzed for 3 h at 105 °C with occasional shaking. This second hydrolysate was taken as labile pool II (LPII) and analyzed for labile pool IIcarbon (LPII-C). Total soil organic C (TOC), LPI-C and LPII-C were analyzed with a TOC analyzer (Multi N/C 2100, Analytik, Jena, Germany). Recalcitrant C (RP-C) was calculated as the difference between the total concentration of the elements (TOC) and the labile pools (LPI and LPII summed together).

At the end of experiment, net N mineralization rates were measured immediately after using a 15 day aerobic laboratory incubation at 23 °C by quantifying the change in 2 M KCl extractable pools of NH_4^+ and NO_3^-. Two 5 g replicates of sieved soil were placed into 15 mL centrifuge tubes. One sample was extracted immediately with 10 mL of 2 M KCl, shaken for 1 h, centrifuged at 3000 rpm and filtered with Whatman no. 1 filter paper. The other sample was incubated for 15 days in the dark prior to extraction. Incubated samples were covered with pierced Parafilm and dampened Kimwipes to maintain soil moisture while allowing for gas exchange. Extracts were frozen prior to analysis. NH_4^+-N and NO_3^--N concentrations were measured colorimetrically by flow injection on a Lachat QuikChem 8000 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA). For each sample, extractable NH_4^+-N and NO_3^--N concentrations were scaled to mg N g $soil^{-1}$ using extract volume, sample mass, and moisture content. Net mineralization rates (N_{min}) were calculated as the change in inorganic N (NH_4^+ and NO_3^-) before and after the 15-d incubation.

We measured the potential activity of five extracellular enzymes that degrade substrates that are known to be common constituents of SOM. These included acid phosphatase (AP), which releases ester-bonded phosphates from soil organic matter, β-1,4-glucosidase (BG), which hydrolyzes cellobiose into glucose, β-1,4-*N*-acetylglucosaminidase (NAG), which breaks down chitin, and peroxidase and polyphenol oxidase (PER and PPO, respectively), which degrade lignin. All assays were run by mixing 1.5 g of soil with 100 mL of 50 mM, pH 5.0, acetate buffer. The suspensions were continuously stirred and twenty-four 200 μL aliquots of the suspension were transferred to 96-well microplates. Microplates were incubated in the dark at 23 °C for 2 h (NAG and AP), 5 h (BG) and 4 h (PER and PPO). NAG, BG and AP activities were measured fluorometrically (excitation, 365 nm; emission, 450 nm) using substrates linked to a fluorescent tag (4-methylumbelliferone), while PER and PPO activities were measured colorometrically using the substrate L-dihydroxyphenylalanine.

2.4. Phospholipid fatty acids (PLFA)

Soil microbial PLFAs were analyzed according to Bossio and Scow (1998). Briefly, PLFAs were extracted, fractionated, quantified, and analyzed in the amount of fresh soil equivalent to 8 g of dry soil. The fatty acid methyl esters of these samples were identified based on chromate graphic retention time using an Agilent 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) and the MIDI Sherlock Microbial Identification (MIDI, Inc., Newark, DE, USA). The fatty acid methyl ester profiles were compared with analytical standard mixtures ranging from C9 to C30 FAME (Microbial ID, Inc.) to determine the mole percentage of PLFAs. Concentrations of each PLFA were calculated relative to 19:0 internal standard concentrations. The fatty acid nomenclature employed in this work was that described by previous studies (Frostegård and Bååth, 1996). For this study, i15:0, a15: 0, i16: 0, i17: 0, a17: 0, cy17: 0, 16: 1ω7c, 16: 1ω9c, and cy19:0 were chosen as bacterial markers, whereas polyunsaturated PLFAs, i.e., 18: 2ω6c and 18: 3ω6c, represented fungi. While the marker 16: 1ω5c is commonly considered a marker for arbuscular mycorrhizal fungi, it also is present in bacteria. Given that our plots had no arbuscular mycorrhizal plants, we decided it would be best to not use treat this lipid as a fungal biomarker. Normal saturated PLFAs, including 15:0, 16:0, and 17: 0 and monounsaturated PLFAs, including 17: 1ω8c, 18: 1ω5c, 18: 1ω9c, and 20: 1ω9c, were considered in assessing the variability of soil microbial community composition (Zhao et al., 2014).

2.5. Calculations and statistics

One-way ANOVA was used to assess the effects of root exudate stoichiometry on the soil response variables for a given forest type. Before analysis, all of the data were tested for normality. If the data were not normally distributed, they were l*n*-transformed before analysis. The statistical tests were considered significant at the

$P < 0.05$ level. Individual comparisons among means were performed using Tukey's pairwise comparison test. All statistical tests were performed with the software Statistical Package for the Social Sciences (SPSS) software, version11.0.3.

3. Results

3.1. Soil total organic C, labile and recalcitrant C pools

At both soils, exudate additions induced similar changes in total organic carbon (TOC), presumably through priming effects, with the greatest losses occurring in soil receiving C alone (approximately 7% loss relative to the controls). However, while exudates consistently decreased TOC, significant treatment effects were only found in both C:N = 100 and C-only (Fig. 1A), suggesting that priming effects were greatest when C was added with little or no N.

Treatment effects on the sum of the two labile C pools (hereafter LP-C) and recalcitrant C pools (RP-C) depended on soil type and C fraction (Fig. 1B and C). In general, exudate additions had no significant effects on LP-C pools, but significantly decreased RP-C pools in the spruce-fir soils. In contrast, exudate additions tended to decrease LP-C pools in the plantation soils, with significant reductions occurring in soils receiving exudates with the least N (i.e., the C:N = 100 and C-only treatments). Moreover, no significant treatment effects on RP-C pool were measured in the plantation soils (Fig. 1C).

3.2. Soil N mineralization rate

Net N mineralization decreased in response to exudate additions in the spruce-fir soils (all five treatments relative to controls), but increased in response to exudates in the plantation

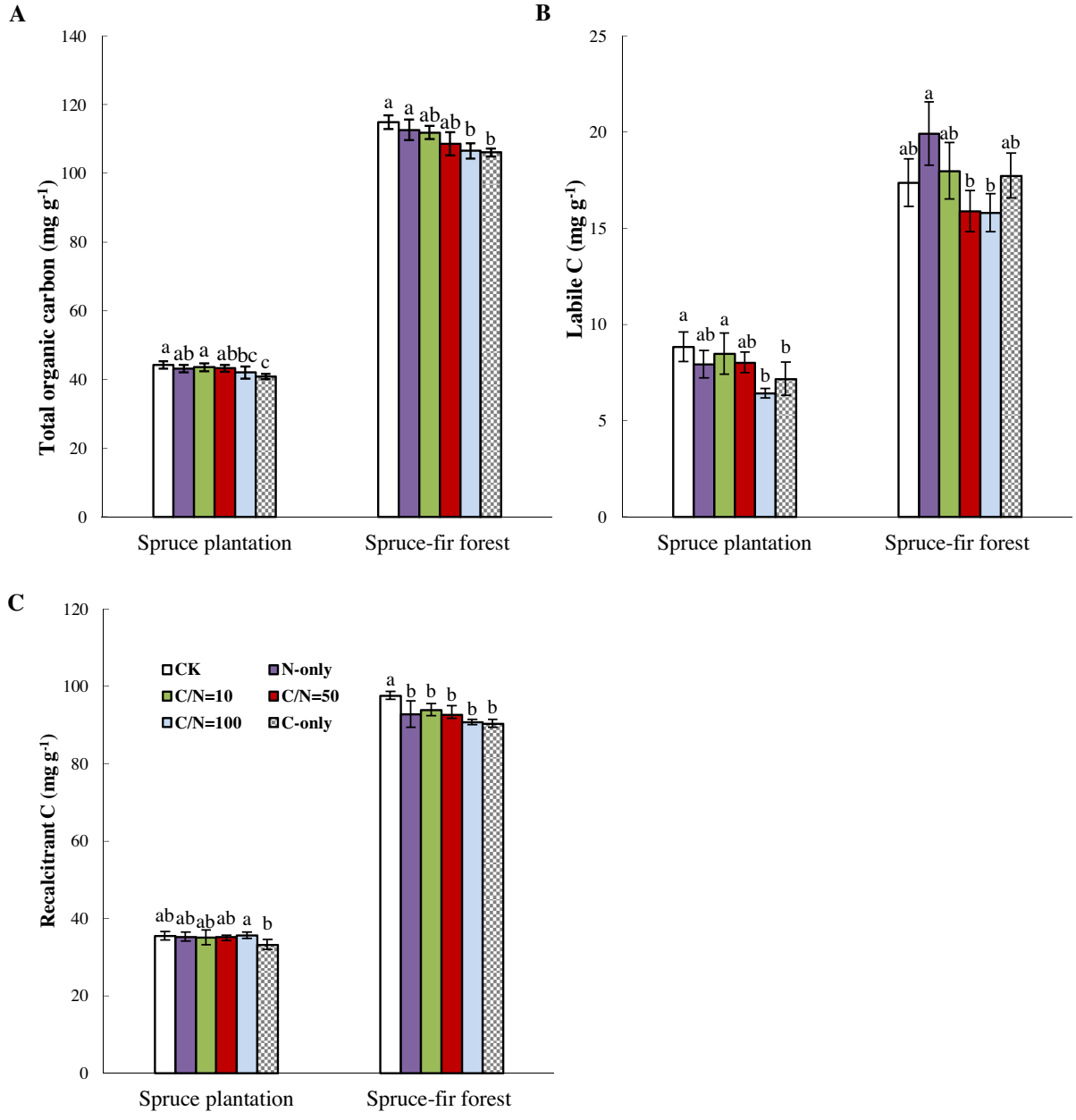

Fig. 1. Effects of simulated root exudates with different C:N stoichiometry on soil total carbon (A), labile C (B), and recalcitrant C pools (C) in the spruce-fir and spruce plantation soils. Values are means ± 1 and different lowercase letters mean significant differences ($P < 0.05$) among treatments at a given soil type.

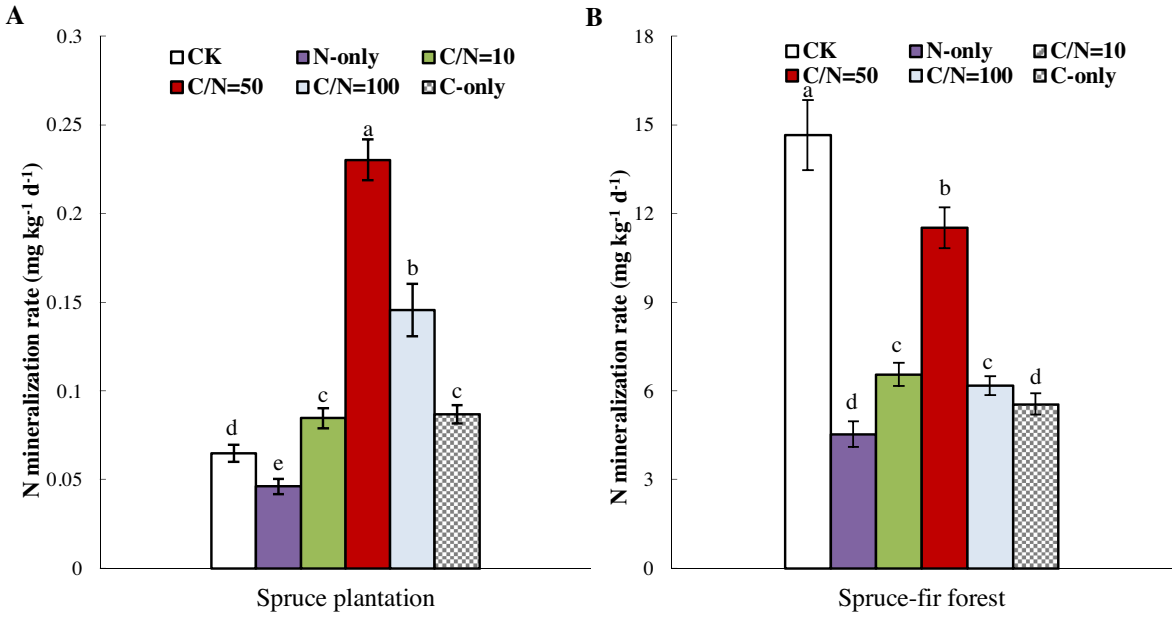

Fig. 2. Effects of simulated root exudates with different C:N stoichiometry on potential N mineralization rate in the spruce-fir and spruce plantation soils. Values are means ± 1 SE and different lowercase letters mean significant differences ($P < 0.05$) among treatments at a given soil type.

soils (four of the five treatments relative to controls; Fig. 2). N-only addition significantly decreased net N mineralization in the plantation soil. In both soils, net N mineralization was greatest in soils receiving exudates that had a C:N of 50.

3.3. Microbial community composition

The response of microbial PLFAs to exudate additions differed between the two soils (Fig. 3). Exudates decreased concentrations of total and bacterial PLFAs in the spruce-fir soils, with the exception of N-only, during which the concentrations of total and bacterial PLFAs were statistically higher compared to the control (Fig. 3A and B). Likewise, exudate addition generally decreased fungal PLFAs concentrations in the spruce-fir soils, but significant treatment effects were measured only in both C:N = 100 and C-only. In contrast, exudate additions significantly increased the concentrations of total, bacterial and fungal PLFAs in the plantation soils, with the exception of N-only, where no statistically significant differences between the treatments were found for all PLFAs (Fig. 3A–C).

Among the treatments, N-only addition significantly increased the bacterial to fungal PLFAs ratios in the plantation soils (Fig. 3D). C addition significantly decreased the bacterial to fungal PLFAs ratios in the plantation soils, except that there was no significant difference between the C:N = 10 and control treatment. In contrast, C addition had no significant effects on the bacterial to fungal PLFAs ratios in the spruce-fir soils (Fig. 3D).

3.4. Extracellular enzyme activity

The responses of extracellular enzyme activity to exudate addition varied greatly with soil type and specific enzyme (Fig. 4). Exudate additions significantly decreased soil acid phosphate enzyme activity (AP) in the spruce-fir forest soils, except in the N-only treatment which significantly increased AP (Fig. 4A). Similarly, exudate additions generally decreased AP activities in the plantation soils, but significant treatment effects were measured only in both C:N = 100 and C-only.

Exudate additions significantly decreased β-N-acetylglucosaminidase (NAG) activities in the spruce-fir soils, with the exception of N-only addition, where no significant treatment effect was found for NAG activity. In contrast to the spruce-fir soils, exudate additions tended to increase NAG activities in the plantation soils, but significant differences were found only in both N-only and C:N = 50 (Fig. 4B).

The response tendency of β-D-Glucose glycosidase (BG) activity to exudate additions was generally similar to AP in both soils. Exudate addition significantly decreased BG activities of the two forest soils, with the exception of N-only in the spruce-fir soils, where no statistically significant differences between the treatments were found (Fig. 4C).

Exudate additions generally decreased soil polyphenol oxidase (PPO) activities in the spruce-fir soils, but significant differences between the treatments were found only in both C:N = 100 and C-only (Fig. 4D). By contrast, exudate additions tended to increase PPO activities of the plantation soils, but significant treatment effects were measured only in C:N = 50, 100 and C-only. Exudate additions all induced significant increases in peroxidase (PER) activities for the two forest soils (Fig. 4E).

4. Discussion

Knowledge of how exudate stoichiometry interacts with soil N availability to influences priming effects and nutrient fluxes is critical for predicting ecological consequences of root-microbe interactions on soil C-balance, particularly in the context of global environmental change. Here the results show that root exudate mimics containing C (but little to no N) induced the greatest losses of soil C. However, despite similarities between the two sites in terms of soil C losses, exudate inputs had divergent effects on SOM fractions, microbial processes and nutrient fluxes. Consequently, the results suggest that exudate-induced priming effects on C-nutrient couplings can be driven by multiple factors, such as the recalcitrance of SOM, N availability and soil microbial communities (Fig. 5).

4.1. Linkages between exudates and priming effects

Numerous empirical and modeling studies have confirmed that root exudates can stimulate SOM decomposition and nutrient

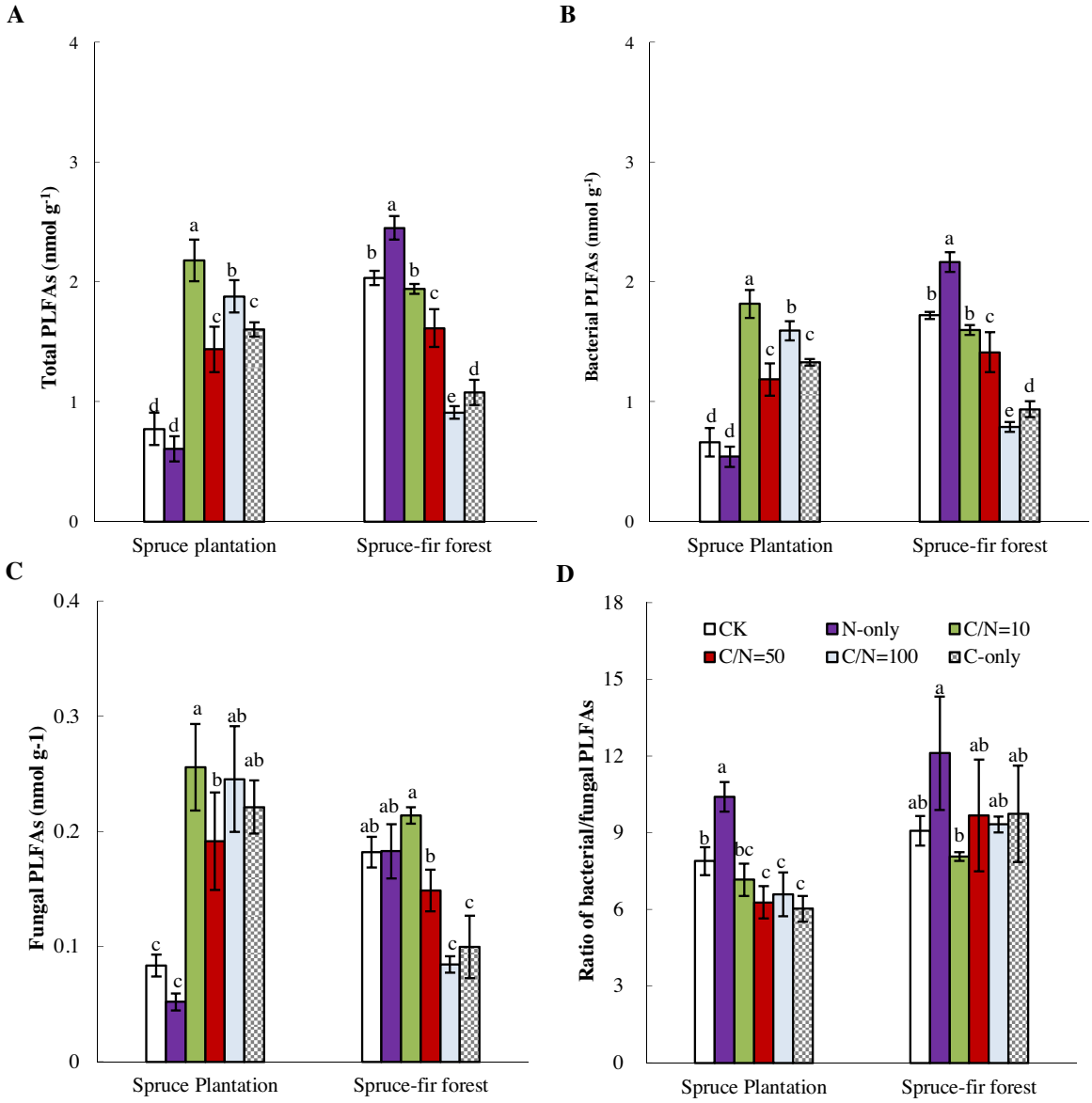

Fig. 3. Effects of simulated root exudates with different C:N stoichiometry on contents of soil microbial phospholipid fatty acid (PLFA) in the spruce-fir and spruce plantation soils. A, total PLFA in microbia. B, bacterial PLFA. C, fungal PLFA. D, bacterial to fungal PLFA ratio. Values are means ± 1 SE and different lowercase letters mean significant differences ($P < 0.05$) among treatments at a given soil type.

release through microbial stimulation (i.e., rhizosphere priming effects; RPE) (Kuzyakov and Domanski, 2000; Fontaine et al., 2003; Cheng et al., 2014; Hafneret al., 2014; Brzostek et al., 2015). The decrease in TOC of two forest soils in response to exudate C mimics shown here experimentally reinforces previous observations, and further support the prevailing paradigm that exudates can accelerate losses of soil C by fueling microbial growth and enzyme synthesis (i.e., co-metabolism).

In this study, the greatest losses of soil C (i.e., positive priming effects) occurred when root exudate mimics contained C (but little to no N), whereas N addition tended to decrease priming effects on SOM decomposition. These results challenge existing theory about the role of exudate chemistry in mediating RPE. Finzi et al. (2015) used a stoichiometrically constrained microbial decomposition model (MCNiP) to show that the largest losses of SOC (i.e., greatest RPE) should occur when the C:N ratio of exudates is the narrowest (i.e., there is ample N in the substrate). Similarly, Drake et al. (2013) using the same MCNiP model and a field experiment, showed that exudates containing N induced stronger RPE than when C was added alone. Given that RPE can be strongly controlled and influenced by soil variables, such as soil texture, structure, chemistry, microbe community or activity (Wutzler and Reichstein, 2013; Cheng et al., 2014), it is possible that the differences in soil properties between the experimental soils may be partly responsible for these conflicting responses. For example, the Harvard Forest soils used in the Drake et al. (2013) study probably contained even lower extractable N than our "low N" plantation soils. Thus, the N supplied via the exudate additions may have enhanced RPE owing to the N-cost of producing extracellular enzymes to degrade the SOM. However, the direct experimental evidence in support of this assumption is scare, and thus, the degree to which soil properties mediate RPE represents a critical area for future research.

4.2. The source of the SOM that gets primed

Despite the large number of RPE studies, there has been limited consideration of the sources or origins of the SOM that actually gets

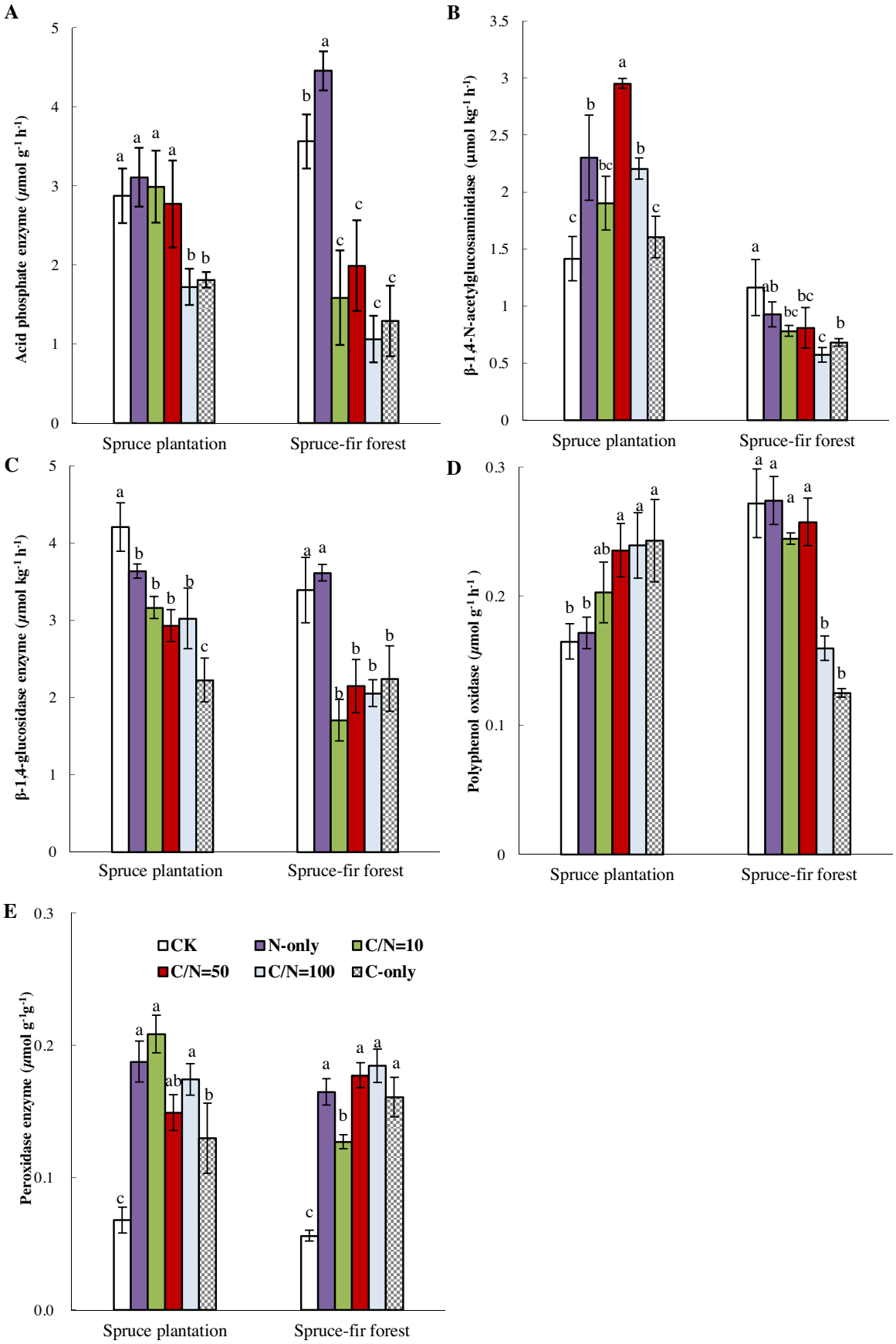

Fig. 4. Effects of simulated root exudates with different C:N stoichiometry on five soil extracellular enzyme activity in the spruce-fir and spruce plantation soils. A, acid phosphatase (AP). B, β-*N*-acetylglucosaminidase (NAG). C, β-D-Glucose glycosidase (BG). D, polyphenol oxidase (PPO). E, peroxidase (PER). Values are means ± 1 SE and different lowercase letters mean significant differences ($P < 0.05$) among treatments at a given soil type.

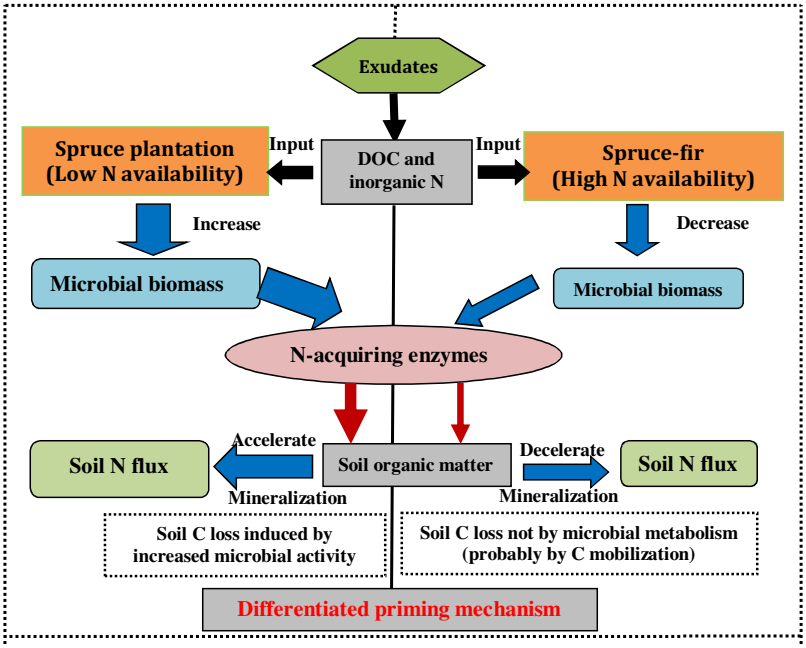

Fig. 5. Conceptual model of C and N fluxes of the two forest soils in response to exudate stoichiometry. In the low N plantation soils (left), microorganisms have great demand for nutrients due to low N availability and thus utilize exudate C as energy source. As a result, microbial biomass and activities increase and thus enhance the production of N-acquiring enzymes to decompose soil organic matter and release N from SOM. In contrast, in the high N spruce-fir soils (right), microorganisms have less demand for nutrients, leading to reduced production of N-acquiring enzymes enzymes and N mineralization rates. The black, blue and red arrow-lines represent the ecological processes mediated by exudate, soil microorganisms, and exoenzymes, respectively. The size of the boxes and arrows indicates a relative magnitude of a flux or intensity of a process. See further explanation in text. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

primed. Using an acid hydrolysis procedure to chemically separate an operationally defined "labile" and "recalcitrant" SOM pools, we found that C additions with low N decreased both fractions in the low N plantation soils but only the recalcitrant fraction in the high N spruce-fir soils. While the mechanism underlying this difference is unclear, a possible explanation relates to differences in how much N is contained in the SOM of each fraction. In the low N plantation soils, microbial N demand is likely to be greater than in the spruce-fir soils, such that the microbes are producing greater amounts of proteolytic enzymes to mine N from this fraction. Additionally, differences in the microbial communites, in terms of their stoichiometry and enzymatic capabilities, could produce similar patterns (Fontaine et al., 2003; de Graaff et al., 2010; Whitaker et al., 2014). It should be noted, too, the observed patterns in soil exoenzyme activities and microbial communities among exudate treatments weren't completely consistent with the response tendency of labile and recalcitrant C sources to exudate stoichiometry. The underlying mechanism about the degree to which exudates stoichiometry mediates microbial processes and soil C fractions warrants further study.

4.3. Different priming mechanism between the spruce-fir and plantation soils

Although exudates induced similar priming effects on soil C losses between the two soils, the underlying mechanisms likely differed. In the low N plantation soils, microbial biomass increased in response to exudate addition, resulting in accelerated N cycling (i.e., increased N mineralization and N-acquisition enzyme activities) and positive priming effects on SOM decomposition. This mechanism is consistent with most microbial/soil C models that predict that positive priming effects are generally accompanied by increases in microbial biomass and activity as microbes mine SOM for nutrients (i.e., a direct biotic microbial metabolic mechanism; Fontaine and Barot, 2005; Blagodatskaya et al., 2007; Drake et al., 2013; Wutzler and Reichstein, 2013; Finzi et al., 2015). However, in the high N spruce-fir soils, positive priming in soils in which the microbial biomass actually decreased. Consequently, N mineralization rates were also reduced. This finding challenges the assumption that exudates induce positive priming effect by stimulating microbial decomposers to mine SOM for N. Recent emerging studies argue that abiotic mechanisms (e.g., C mobilization and biological accessibility) also play an important role in driving priming effects on SOM decomposition (Kemmitt et al., 2008; Schmidt et al., 2011). For example, exudate inputs can liberate C from protected mineral-organic associations, and thereby enhance microbial access to previously mineral-protected SOM compounds (Keiluweit et al., 2015). Previous studies have reported that priming can be controlled by the degree to which SOM in conifer forests is protected by aluminosilicates containing oxalate-extractable iron (Rasmussen et al., 2007) but whether this mechanism controlled priming responses in the spruce-fir soils of this study is unknown. In addition, the results of bacterial: fungal dominance in response to exudate additions to some extent supported our assumption that the exudate-induced priming effects on soil C losses of the two forest soils may be driven by different mechanisms. For example, C addition decreased the bacterial to fungal PLFAs ratios only in the plantation soils as fungi have lower nutrient requirements than bacteria (Högberg et al., 2008). However, recent evidence also demonstrated that the changes in specific populations (i.e., gram positive bacteria) rather than the whole microbial biomass and/or microbial community may be major controllers of exoenzyme production and subsequent priming events (Bird et al., 2011; Wang et al., 2015). Future investigations will be needed to validate this indirect priming mechanism and quantify the relative contribution of direct and indirect mechanism to priming effects in future research, such as direct biotic microbial metabolic mechanism, indirect C mobilization mechanism or blending of two of them for a given ecosystem.

The ecological significance of priming effects demonstrated here should be considered with caution given some caveats associated with the experimental system. First, an inorganic source of N (NH_4Cl) was used in the N-only treatment but an organic N source was used in the C:N exudate treatments. Because amino acids contain C, there is no way to add amino acids to soil without also adding C. While NH_4Cl could have been used to make the C:N substrates (rather than amino acids), such a treatment would not reflect the types of N compounds released by roots, which are mostly amino acids (Phillips et al., 2006). Consequently, the results of the C-only and N-only treatments cannot be compared meaningfully with those containing both C and N, as C content is confounded with N content. A second limitation of the experimental system is that exudate additions were provided in large pulses one time per day) as opposed to more continuously, like what be found in a real rhizosphere. Given that it would be impossible to create this type of condition experimentally, the exudates were pulsed semi-continuously instead. While there is little reason to think this would matter, we can't rule out that this could have some potential effects on the microbes. However, any such effects would presumably negligible over the course of a 50 day experiment. Third, exudate compounds were delivered to disturbed soils in the lab that are not subject to the full suite of biotic and abiotic factors that occur in the field. Although not without significant drawbacks, this approach is currently the most effective method for studying the effects of exudates on soil biogeochemical processes (Keiluweit et al., 2015).

In conclusion, this study demonstrates that root exudate mimics containing C (but little to no N) induced the greatest losses of soil C at both sites. However, despite the uniformity between the two sites in promoting soil C losses, the exudate inputs have divergent effects on SOM fractions, microbial biomass and activities, and nutrient fluxes. For example, in plantation soils, exudate addition increased microbial biomass (especially fungi), accelerated N cycling (N mineralization and N-acquisition enzyme activities) and increased lignin-degrading enzyme activities relative to controls. In contrast, exudate additions to spruce-fir soils decreased microbial biomass, decelerated N cycling, accompanying with variable impacts on lignin-degrading enzyme activities (decreased phenol oxidase but increased peroxidase). These results suggest that exudate-induced priming effects on soil C-nutrient couplings may be co-driven by multiple mechanisms. The results provide additional evidences toward a robust theoretical foundation for better understanding the ecological consequences of exudate stoichiometry on soil C-nutrient cycling in forests.

Authors contributions

Q.L., R.P.P. and H.J.Y. conceived the idea for all experiments; R.B. L., Z.F.X., Q.L. and H.J.Y. performed the research and analyzed the data; and R.P.P. and H.J.Y. wrote the paper.

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