Exudate components exert different influences on microbially mediated C losses in simulated rhizosphere soils of a spruce plantation

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Abstract
Background and aims Root exudates play a vital role in driving ecosystem carbon (C) cycling; however, few studies have examined the degree to which a specific exudate component affects soil C loss. The objective was to examine the impacts of different exudate components on microbially-mediated C decomposition and their underlying mechanisms.

Methods In a well-controlled simulated rhizosphere system, we added exudate chemicals (glucose, glycine and oxalic acid) to spruce (Picea asperata) plantation soils over a 35-day period. The total C contents, net N mineralization rates, microbial communities and extracellular enzymes were measured.

Results The three exudate components induced different C losses by different mechanisms. Oxalic acid promoted net C loss by accelerating microbial mineralization of soil organic matter (SOM). In contrast, glucose resulted in a net C accumulation, which challenged the assumption that glucose serves as a co-metabolite in driving SOM decomposition to lose C. Glycine increased the total C content via negative priming effects.

Conclusions Exudate-induced rhizosphere priming effects are not entirely dependent on the energy properties of root exudates. Different exudates may affect SOM decomposition differently, thus the component-specialized rhizosphere processes induced by individual exudate components on soil C dynamics should be integrated into forest C cycle-climate feedbacks under environmental changes.

Keywords Exudate · Soil microbial community · Extracellular enzyme · Carbon loss · Subalpine coniferous forest

Introduction
Root exudates are mostly organic compounds that are secreted by plant roots and mycorrhiza hyphae, which
include sugars, organic acids, amino acids, phenolics and other secondary metabolites (Haichar et al. 2014; van Hees et al. 2005). The total amount of exudates released into the soil accounts for approximately 17% of the total photoassimilate C across varieties of plant species (Nguyen 2003). These exudates have multiple ecological effects on the belowground ecosystem, such as regulating the soil microbial community (Koranda et al. 2011; Phillips et al. 2011), changing the chemical and physical properties of the soil (Dakora and Phillips 2002; Keiluweit et al. 2015) and stimulating SOM decomposition (Kuzyakov et al. 2007). Currently, root exudates and their regulation in soil C-nutrient cycling are increasingly being recognized as an important issue affecting the underground ecological processes, especially in the rhizosphere (Haichar et al. 2014; Finzi et al. 2015; Keiluweit et al. 2015).

Both biotic factors (e.g., plant species) and abiotic factors (e.g., elevated CO2 and warming) have been shown to alter the quantity and composition of exudates (Haller and Stolp 1985; Grayston et al. 1997; Wang et al. 2006; Fransson and Johansson 2010; Wu et al. 2012; Bowsher et al. 2016). For example, the exudation of total low-molecular-weight organic acids, amino acids and dissolved monosaccharides was increased by 120–160%, 250% and 130–270%, respectively, under elevated CO2 (Johansson et al. 2009). Under aluminium stress, some original secondary metabolites of exudates from wheat gradually disappeared, and some new secondary metabolites (e.g., N-methyl phenylethylamine and hexatriacontane) were simultaneously excreted (Wang et al. 2006). Consequently, altered exudation patterns may exert important influences on the rhizosphere processes, as well as on terrestrial C cycling (Xu and Chen 2006; Heimann and Reichstein 2008). Thus, elucidating the mechanisms of how certain components of exudates mediate soil biogeochemical processes are of great importance to predict terrestrial C stocks under climate change and other environmental shifts.

Efforts have been made to examine the influences of root exudates on soil biogeochemical processes (Hamer and Marschner 2005; Brzostek et al. 2013; Strickland et al. 2015). However, most previous studies addressing exudate influences have mainly focused on the total rhizosphere C fluxes (Phillips et al. 2009; Koranda et al. 2011; Yin et al. 2013; Yin et al. 2014; Wang et al. 2016) and exudates induced priming effects (i.e., the change in SOM decomposition after fresh organic matter being applied to the soil) (Hamer and Marschner 2005; Brzostek et al. 2013; Strickland et al. 2015). Up to now, the specific ecological effects of different components of root exudates and their underlying regulation mechanisms have rarely been studied, which greatly hinders our ability to predict the ecological consequences of root-microbe interactions on soil C stocks, particularly in the context of global changes.

Hence, the objective of this study was to examine the impacts of three common components of root exudates (glucose, glycine and oxalic acid) on microbiologically-mediated C decomposition in the soils of a dragon spruce (Picea asperata) plantation; these components were chosen because they are common low-molecular-weight organic compounds (Smith 1976), and each of them is representative of one of the three most abundant exudate classes - sugars, amino acids and organic acids (Jones et al. 2003; Watt 2009). There are differences among these three components in the energetical efficiency for microbes. Specifically, glucose is a bioenergetically more favourable sugar to microbes, oxalic acid is of limited bioenergetic use, and glycine has the lowest microbial efficiency (Bradford et al. 2013; Frey et al. 2013; Keiluweit et al. 2015). Consequently, we hypothesized that glucose would act as a co-metabolite, and thus enhance microbial processes and lead to a greater soil C loss by means of co-metabolism (i.e., mineralization of SOM during growth of microbes on a bioavailable C and energy source, see Horvath 1972). In contrast, glycine would have little effect on microbial processes and community composition, which would result in small changes in C content. Oxalic acid may interact with minerals and form the precipitation of short-range ordered aluminium and iron oxides, which may differ in physical and chemical composition and reactivity to organic compounds (Cristofaro et al. 2000). It was also expected to exhibit a positive response on soil C loss. We further hypothesized that the component differences in exudates would result in different rhizosphere priming effects. To test these hypotheses, total C, microbial communities and related extracellular enzyme activities, and the net N mineralization rates in the rhizosphere soils were comprehensively evaluated to explore the ecological consequences and the underlying mechanisms of different exudate components on microbially-mediated C losses in forest soils.
Material and methods

Study site and soil sampling

The study site is located at the Miyaluo Experimental Forest of Lixian County, Eastern Tibetan Plateau (31°35′N; 102°35′E; 3150 m a.s.l.). There are some mosses and grasses (e.g., Carex capilliformis, Deyeuxia arundinacea, Festuca ovina) covering the forest floor. The spruce plantation originated from clear-cut land in 1950s; no management practice was added (Xu et al. 2010). The soils are classified as a Cambic Umbrisols (Yin et al. 2016). To investigate the impacts of root exudates on biochemical cycling at different soil layers, we collected soil from the organic horizon and the top 15-cm of the mineral layers from the spruce plantation in August 2015. After removing the recent litter and decomposing litter, over one hundred soil samples were randomly sampled with a soil corer (4.7 cm in diameter) to a 20-cm depth. Each soil sample was hand-divided into the organic horizon and the top 15-cm of the mineral soil (hereafter known as the mineral horizon). The organic horizon was distinguished from the mineral horizon by its morphology (including soil color, texture, and consistency; about 5 cm). Each composite soil horizon sample was thoroughly mixed, passed through a 2-mm sieve, and any visible residue was carefully removed manually. Then the sample was divided into two subsamples. One subsample was stored at field moisture at 4 °C for measuring soil physical, chemical and biological properties (Table 1). The other subsample was processed for the exudate addition experiments.

Simulated root exudate experiment

Experimental incubations were carried out in microcosm systems that were made to deliver exudate solution through an artificial root into the soils. The microcosm system was modified from Keiluweit’s system (2015) to obtain sufficient amount of rhizosphere soils for testing (Supplementary Fig. S1). Three microporous cylindrical artificial roots (membrane pore size: 0.12–0.18 μm, length = 100 mm, diameter = 2.5 mm, Rhizosphere Research Products, Netherlands) were equidistantly plugged in a microcosm frame. The microcosm frame consists of a 140 × 100 × 10 mm clear acrylic frame with a 130 × 90 × 8 mm opening, and an acrylic front panel attached for protection. Soils were pre-incubated at 75% field capacity for one week and then packed into the microcosm at field bulk density.

Root exudates were simulated using three common components of root exudates: glucose, oxalic acid and glycine (purchased from Sigma-Aldrich international GmbH, America). Each exudate solution normalized on a C-basis was injected to soils via the artificial roots at the root surface-normalized rate, which mimicked the natural exudation rate of the root area (i.e., 5 μg C m⁻² root area h⁻¹) (Yin et al., 2013; Li et al. 2014), that is, at a rate of 942 μg d⁻¹ per artificial root (with an area of 7.85 cm²). The preliminary experiment of water counterbalance demonstrated that soil water losses through evaporation in each microcosm were counterbalanced by the supply of 1.2 mL solution or deionized water per day during the experimental period. Thus, the packed soil in each microcosm received 1.2 mL root exudate solution (0.4 mL from each artificial root) once a day to guarantee the maintenance of soil water content. Control specimens were treated in the same way using deionized water. To minimize microbial contamination, each exudate solution was filtered through a 2.2 μm membrane and re-prepared weekly. Incubations were conducted in an environmental chamber at 25 °C and 75% relative humidity in the dark for 35 days.

There are four treatments (3 exudate components and the control) for a given soil horizon, and each treatment includes twelve microcosms. Because two soil horizons were involved in the experiment, there are 96 microcosms (2 soil horizons × 4 treatments × 12 microcosms) in total in this study. After the incubation, microcosms

| Table 1 | Selected soil properties in the organic and mineral horizons of the Picea asperata plantation |
|---------|---------------------------------------------------------------|
| Soil properties | Spruce plantation |
|                  | Organic horizon | Mineral horizon |
| Soil pH           | 6.13 (0.09)  | 6.35 (0.10)    |
| Soil electrical conductivity (μS cm⁻¹)⁴ | 147 (7.4)   | 89 (3.6)      |
| Total C (g kg⁻¹)⁵ | 48 (2.1)     | 21 (1.5)       |
| Total N (g kg⁻¹)⁶ | 3.73 (0.15)  | 2.03 (0.15)    |
| C: N ratio       | 12.8 (0.26)   | 10.2 (0.42)    |

All the results are the means (± SE) of three replicates (n = 3)

a Measured in 1:5 soil:water suspensions using a DD-307 conductivity apparatus

b Measured by direct combustion on an elemental analyser (Multi N/C 2100, Analytik, Jena, Germany)
were opened and soils of 0–4 mm on both sides of the root were carefully collected. The soils were viewed as the rhizosphere soil based on the preliminary experiment showing that the exudate solutions were diffused approximately 4 mm away from the artificial root. Given that the amount of rhizosphere soil in each microcosm was insufficient for the analyses of the variables listed below, samples from four parallel microcosms were thoroughly mixed and regarded as a composite, and twelve microcosms from each treatment were randomly divided into three composites (i.e., three replicates for each treatment). The mixed samples of each replicate were used for the measurement of pH, net N mineralization rate and extracellular enzyme activities or stored at −80 °C for PLFAs extraction or air-dried for the determination of soil total C.

Total C, pH and net N mineralization

Total C in soils was measured by dry combustion (Multi N/C 2100, Analytik, Jena, Germany). Soil pH was measured in 1: 2.5 soil: water suspensions with a pH electrode. Net N mineralization rates were measured using a 15-d aerobic laboratory incubation at 25 °C by quantifying the change in 2 M KCl extractable pools of inorganic N. Inorganic N was extracted with a 2 M KCl extractant (soil: extractant = 1:5), and the concentration of inorganic N in the extract was measured colorimetrically by flow injection using an AutoAnalyser III (SEAL Analytical, Germany).

Extracellular enzymes assay

The potential activities of enzymes that hydrolyse cellobiose into glucose (β-1,4-glucosidase (BG)), degrade chitin (β-1,4-N-acetyl-glucosaminidase (NAG)), and degrade lignin (peroxidase (PER)) were assayed based on a modification of previous methods (Saiya-Cork et al. 2002). Briefly, all the assays were run by mixing 2 g of fresh soil with 100 mL of 50 mM sodium of acetate buffer (pH = 5.0). The suspensions were continuously stirred and twenty-four 200 mL aliquots of the suspension were transferred to 96-well microplates. The microplates were incubated in the dark at 23 °C for 2 h (NAG), 5 h (BG) and 4 h (PER). BG and NAG activities were measured fluorometrically (excitation, 365 nm; emission, 450 nm) using substrates linked to a fluorescent tag (a 50 μL aliquot of 100 μM 4-methylumbelliferone to each sample well), while PER activities were measured colorimetrically (460 nm) using substrate of a 50 μL aliquot of 25 μM L-dihydroxyphenylalanine to each sample well. The Thermofisher Multimode Reader (Varioskan Flash, Thermo, USA) was used as microplate reader to assess the enzyme activities.

Phospholipid fatty acids

Phospholipid fatty acids (PLFAs) were extracted, fractionated and methylated with minor modifications using the method of Zelles (1997). PLFA samples were dissolved in hexane and analysed using an Agilent 6850 Gas Chromatograph (30-m capillary column). Peaks were detected by flame ionization, quantified by the Agilent 3398 Chemstation software, and identified by reference to a bacterial acid methyl ester mix (BAME, Supelco). In the community analysis, thirteen microbial PLFA biomarker were assigned to 6 microbial categories: Gram-positive (+) bacteria (15:0i, 15:0a, 16:0i, 16:0a, 17:0i, 17:0a), Gram-negative (−) bacteria (16:1ω7, 18:1ω7), fungi (18:2ω6,9, 18:1ω9), actinomycetes (16:010 Me, 18:010 Me), and protozoa (20:4ω6,9) (Zelles 1997, 1999; Brante et al. 2006). Unassigned PLFAs (14:1i, 14:0, 15:1i, 15:0, 16:1ω5, 18:1ω5) were included in total PLFA yields and all community analyses procedures. The ratios of bacteria to fungi were calculated as fungal group PLFAs yield divided by the sum of Gram (+) bacteria and Gram (−) bacteria PLFA groups (Zelles 1999).

Calculations and statistical analysis

We quantified treatment effect as the change of a given response variable between the exudate component treatment and the control sample [i.e., (variableexudate component - variablencontrol)/variablencontrol]. Mean treatment effect (MTE) is the average value of all five variables including total PLFA content, BG, NAG, PER and net N mineralization rate. One-way ANOVA was performed to assess the effects of exudate components on total soil C, pH, microbial communities, enzymes and the net N mineralization rate for a given soil horizon (Table 2). Two-way analysis of variance (ANOVA) was also used to examine the effects of exudate components, soil horizons, and their interactions on the changes (relative to the control) in total soil C, microbial communities, enzymes and the net N mineralization rate (Table 3).
Before analysis, all data were tested for the assumptions of ANOVA. Data of actinomycetic and protozoal PLFAs of the organic horizon and fungal PLFAs of both organic and mineral horizon were found to be heterogeneous, which were ln-transformed before analysis. The statistical tests were considered significant at the $P < 0.05$ level. Statistical analyses were performed in SPSS version 19.0 (SPSS Inc., Chicago, USA).

Table 2 Results of one-way ANOVA analyses of total soil C, pH, microbial communities, enzymes and the net N mineralization rate among the control and three exudate components

| Variables                      | d.f. | Organic horizon | Mineral horizon |
|--------------------------------|------|-----------------|-----------------|
|                                |      | $F$             | $P$             | $F$             | $P$             |
| Total C                        | 3    | 18.022          | 0.001           | 6.161           | 0.018           |
| pH                             | 3    | 11.252          | 0.003           | 8.178           | 0.008           |
| Microbial communities (nmol g$^{-1}$) |      |                 |                 |                 |
| Total PLFA                     | 3    | 56.97           | 0.000           | 16.762          | 0.001           |
| Total PLFA                     | 3    | 56.97           | 0.000           | 16.762          | 0.001           |
| Actinomycetic PLFA             | 3    | 18.348          | 0.001           | 36.15           | 0.000           |
| Fungal PLFA                    | 3    | 11.582          | 0.003           | 13.492          | 0.002           |
| Protozoal PLFA                 | 3    | 13.308          | 0.002           | 27.393          | 0.000           |
| Gram(+) PLFA                   | 3    | 4.693           | 0.036           | 18.53           | 0.001           |
| Gram(+) PLFA                   | 3    | 4.693           | 0.036           | 18.53           | 0.001           |
| Bacteria / fungi               | 3    | 28.942          | 0.000           | 2.228           | 0.162           |
| Enzymes (nmol g$^{-1}$ h$^{-1}$) |      |                 |                 |                 |
| β-1,4-glucosidase              | 3    | 4.612           | 0.037           | 32.069          | 0.000           |
| β-1,4-N-acetyl-glucosaminidase | 3    | 4.904           | 0.032           | 1.972           | 0.197           |
| Peroxidase                     | 3    | 6.467           | 0.016           | 17.853          | 0.001           |
| Net N mineralization rate (mg kg$^{-1}$ day$^{-1}$) | 3 | 20.726 | 0.000 | 35.292 | 0.000 |

Table 3 Results of two-way ANOVA (exudate × horizon) on microbial communities, enzymes and the net N mineralization rate (Nmin) related to C cycling

| Source       | d.f. | Total C | Total PLFA | Actinomycetic PLFA | Fungal PLFA |
|--------------|------|---------|------------|--------------------|-------------|
|              |      | $F$     | $P$        | $F$                | $P$         | $F$         | $P$         |
| Exudate      | 2    | 22.382  | 0.000      | 86.151             | 0.000       | 54.641      | 0.000       |
| Horizon      | 1    | 3.555   | 0.084      | 1.672              | 0.220       | 5.919       | 0.032       |
| E × H        | 2    | 0.753   | 0.492      | 15.171             | 0.001       | 0.162       | 0.853       |
| Error        | 12   |         |            |                    |             |             |             |
| Protozoal PLFA | 2    | 28.78   | 0.000      | 21.496             | 0.000       | 73.993      | 0.000       |
| Gram(+) PLFA | 2    | 29.81   | 0.000      | 1.277              | 0.281       | 6.816       | 0.023       |
| Gram(−) PLFA | 2    | 1.846   | 0.200      | 3.453              | 0.065       | 19.199      | 0.000       |
| Bacteria/fungi| 2    | 28.942  | 0.000      | 2.228              | 0.162       | 2.338       | 0.097       |
| Exudate      | 2    | 28.78   | 0.000      | 21.496             | 0.000       | 73.993      | 0.000       |
| Horizon      | 1    | 29.81   | 0.000      | 1.277              | 0.281       | 6.816       | 0.023       |
| E × H        | 2    | 1.846   | 0.200      | 3.453              | 0.065       | 19.199      | 0.000       |
| BG           | 2    | 9.838   | 0.003      | 4.754              | 0.030       | 4.173       | 0.042       |
| NAG          | 2    | 1.559   | 0.236      | 0.486              | 0.499       | 3.206       | 0.099       |
| PER          | 2    | 0.986   | 0.401      | 0.918              | 0.426       | 0.557       | 0.587       |
| Nmin         | 2    | 9.838   | 0.003      | 4.754              | 0.030       | 4.173       | 0.042       |
| Exudate      | 2    | 9.838   | 0.003      | 4.754              | 0.030       | 4.173       | 0.042       |
| Horizon      | 1    | 1.559   | 0.236      | 0.486              | 0.499       | 3.206       | 0.099       |
| E × H        | 2    | 0.986   | 0.401      | 0.918              | 0.426       | 0.557       | 0.587       |
Results

Total C content

The three exudate components led to different soil C losses across the two soil horizons (Fig. 1). Compared to the control, glucose and glycine additions generally increased the total C contents (Fig. 1, Table 2) and the magnitude of this increase depended on soil horizons (Table 3). Total soil C in the organic horizon increased by 3% and 2% with the addition of glucose and glycine, respectively whereas that in the mineral horizon were increased (by 2%) only by glucose. In contrast, the addition of oxalic acid significantly decreased ($P < 0.05$) the total soil C by 2% in both horizons of the spruce plantation.

Soil pH

The response of pH to exudate addition varied among the exudate components across the two soil horizons (Fig. 2). Compared to the control, glycine significantly ($P < 0.05$) lowered the pH by 0.26 and 0.42 units in the organic and mineral horizon, respectively, whereas the pH was not affected by glucose or oxalic acid addition in either horizon after 35 days of incubation (Fig. 2, Table 2).

Net N mineralization and soil enzyme activity

Net N mineralization rates differed considerably among the three exudate components (Fig. 3). Net N mineralization rates were significantly increased by oxalic acid additions but decreased by glycine addition compared to the control ($P < 0.05$; Table 2). The addition of glucose had no remarkable influence on the net N mineralization rates in both horizons of the spruce plantation.

Extracellular enzyme activity responded to exudate additions differently among exudate components across the soil horizons (Fig. 4). Compared to the control, glucose and oxalic acid additions generally increased the extracellular enzyme activities with the exception of NAG activity in the organic horizon soil (Fig. 4b). In contrast, glycine additions slightly decreased BG activity, but had no influence on the activity of NAG and PER in either horizon (Fig. 4).

Microbial community

The three common exudate components had divergent effects on microbial PLFAs across soil horizons (Fig. 5, Table 2). Glucose generally increased the concentrations of total PLFAs, actinomycetes, protozoa, fungi and Gram (+) bacteria in the rhizosphere of both soil horizons (Fig. 5). Similarly, oxalic acid addition resulted in a significant increase ($P < 0.05$; Table 2) in PLFAs concentrations of actinomycetes, protozoa, fungal, Gram (−) bacteria and Gram (+) bacteria (Fig. 5a - d). Glycine additions significantly decreased the total PLFAs, actinomycetes, protozoa, fungal and Gram (−) bacterial PLFAs (Fig. 5a - e).
but did not affect the Gram (−) bacterial PLFAs and the ratios of bacteria to fungi (Fig. 5f–g).

Treatment effects of different exudate components on microbial processes

The effects of different exudate components on microbial activity, enzyme activity and SOM decomposition are presented in Fig. 6. In general, oxalic acid had greater positive effects on the total PLFAs (except in the mineral horizon), enzymes of BG, NAG and PER and net N mineralization rate compared to glucose, while glycine had negative effects on these enzymes (with the exception of NAG in the organic horizon and PER in the mineral horizon). The magnitude of these effects depended on soil horizon (Fig. 6, Table 3). In the organic horizon, oxalic acid addition enhanced the total PLFAs, BG, NAG, PER and net N mineralization rate by 20%, 20%, 15%, 12% and 15%, respectively, with a MTE of 17%. The increases induced by glucose were
Fig. 4 Extracellular enzyme activities (a) β-1,4-glucosidase, (b) β-1,4-N-acetylglucosaminidase, (c) Peroxidase of the rhizosphere soils originated from the organic and mineral horizons of the Picea asperata plantation. Different lowercase letters in the same horizon indicate significant differences among exudates at $P < 0.05$. All the results are the means (± SE) of three replicates ($n = 3$).
13%, 19%, 0, 10% and 3%, respectively, with a MTE of 10%. However, glycine addition suppressed these variables (except NAG) by 20%, 3%, 3% and 54%, respectively, with a MTE of 15%. In the mineral horizon, these variables were enhanced by 9%, 18%, 13%, 34% and 47%, respectively, with a MTE of 24% under the addition of oxalic acid. The increases induced by glucose were 16%, 18%, 4%, 21.82% and 22%, respectively, with a MTE of 16%. However, these variables (except PER) were suppressed by glycine addition. The magnitudes of these suppressions were 3%, 26%, 7% and 10%, respectively, with a MTE of 9% in contrast to the control (Fig. 6).

Discussion

Despite the fact that rhizosphere soil occupies a relatively small percentage of the total soil volume, rhizosphere processes are quantitatively important components of terrestrial C and nutrient cycles (Finzi et al. 2015). Understanding rhizosphere C losses induced by different exudate components and their underlying mechanisms are critical for predicting ecosystem C stock responses to global changes. In this study, we found that different exudate components induced divergent effects on soil C decomposition through the obvious changes of microbial activities and processes. Glucose addition resulted in an accumulation of soil C with increased microbial activities that was unrelated to SOM degradation. Glycine caused an increase of soil total C via decreased microbial activities and inhibited microbial processes. Meanwhile, the addition of oxalic acid led to a net C loss through enhancing microbial activities and process that accelerated SOM degradation.

Different priming effects induced by the three exudate components

The priming effect is widely believed to result from changes in SOM decomposition in response to exogenous C additions (Kuzyakov 2002). However, the directions and magnitudes of rhizosphere priming effects induced by different exudate C inputs remained controversial (Cheng 2009; Dijkstra et al. 2013). Positive, negative and neutral rhizosphere priming effects have been reported in previous studies (Hamer and Marschner 2005; Dijkstra et al. 2013; Wang et al. 2016). In the present study, we assumed that the addition of bioenergetically more favourable glucose would cause greater C losses via a stronger priming effect than that of less favourable oxalic acid. However, unexpected results were observed; by shifting the microbial community structure, accelerating microbial activities and related processes (Figs. 3, 4 and 5), oxalic acid induced a positive priming effect that resulted in a net C loss (Fig. 1). In contrast, soil receiving glucose led to significant higher microbial activities of protozoa, actinomycetes and Gram (-) bacteria (Fig. 5a–f), but no significant increase ($P > 0.05$) in the net N mineralization rate (Fig. 3) and no loss of total soil C were observed (Fig. 1). This interesting result challenged the traditional assumption that exudates serve as co-metabolites in driving microbial decomposition of SOM to promote C loss (Bais et al. 2006; Philips et al. 2006). The increased microbial activities after fresh C inputs with little SOM decomposed were described as an apparent priming effect by Blagodatskaya et al. (2007, 2008). Presumably, after glucose is added to soils, microbes switch from decomposing recalcitrant SOM to utilizing glucose for their C and energy requirements. According to Fontaine et al. (2003), the most assimilable compounds are used by r-strategist microorganisms that only decompose fresh organic compounds. Ongoing work in our laboratory is aimed at applying isotope labelling techniques such as $^{13}$C to further prove the apparent priming effect that really occurred after input the amount of glucose in this study.

The addition of glycine caused a negative priming effect resulting in an increase in total soil C content (Fig. 1). The negative priming effect was presumably attributed to the following reasons. First, the decreased soil pH resulted from glycine addition. In the present study, the pH value in soil amended with glycine was decreased by 0.26 and 0.42 units in organic horizon and mineral horizon, respectively (Fig. 2). As a result, the decreased pH would reduce microbial biomass and activity (Rousk et al. 2009), which would induce a negative priming (Wang et al. 2016). Second, the remarkable higher N concentration (data not shown) caused by glycine. The high accumulation of N nutrient fully satisfied microbial N requirement, and thus there is no need for microbes to produce enzymes (e.g., $\beta$-1,4-N-acetyl-glucosaminidase and peroxidase) to access additional N, which might result in a suppression of microbial mineralization of SOM as indicated by N mining theory (Dijkstra et al. 2013).
Microbial community and processes

Soil microbes and related enzymes are involved in many processes known to affect biogeochemical cycling, which depend on root exudates in the rhizosphere (Bird et al. 2011; Haichar et al. 2014). Results from our study clearly showed that microbial communities, extracellular enzyme activities and net N mineralization rates differed considerably in driving C decomposition among exudate components. Previous studies have revealed that labile exudates delivered to the rhizosphere can accelerate SOM degradation by fuelling microbial growth and enzyme production (Fontaine et al. 2003; Dijkstra et al. 2013). However, the obtained results were not completely consistent with the above mechanism. We found that glucose slightly influenced the net N mineralization rate (Fig. 3), although it led to a significant increase in microbial activities (e.g., protozoal PLFA, actinomycetic PLFA, Gram (−) PLFA and total PLFAs) (Fig. 5). Glucose can serve as the main source of C utilized by a wide range of microbial populations (Paterson et al. 2007). After the addition of glucose to the soils, many specialized microorganisms (e.g., r-strategist) grow quickly and only decompose the easily assimilable glucose (Fontaine et al. 2003). Besides, k-strategists microorganisms may also switch from SOM degradation to glucose uptake (Blagodatskaya et al. 2007). It has been further proved that if the added glucose saturated microbial utilization capacity, the absence of a positive priming effect would be observed in many soil types (Paterson and Sim 2013). In the present study, the amount of glucose added to the soils may, to some extent, satisfy the C and energy requirements for microbial growth, and there is no need for microbes to decompose SOM for additional nutrient. As a result, the glucose-C was utilized for increasing microbial C turnover instead of decomposing SOM, and thus the net N mineralization rate would be slightly influenced. Glycine, a type of the amino acid, functions as a nutrient source but with strictly limited energy. Its addition suppressed microbial community activities (Fig. 5). Two potential explanations can be proposed for this result. One is that microbial utilization of glycine was minimal through the microbial community and its incorporation of glycine into microbial biomass was just nearly a third of glucose (Paterson et al. 2007; Bradford...
et al. 2013). The other is that the markedly higher N accumulation in the rhizosphere soil resulted from glycine addition may be toxic to some microorganisms, as high amounts of N accumulation results to high amounts of ‘browning precursors’ (Fog, 1988). As a result, the decreased activity of microbes led to the decrease of SOM mineralization (Fig. 6).

Inconsistent with Strickland et al. (2015), we found that soil receiving oxalic acid exhibited a significant greater microbial biomass accumulation and higher enzyme activity, which accelerated microbial decomposition of SOM (Fig. 6). This interesting phenomenon may be due to several reasons as follows. First, the root-derived oxalic acid acts as chemoattractant signals to microbes, and thus plays a role in regulation of the soil microbial community in the immediate vicinity of roots (Haichar et al. 2014). Second, oxalic acid can provide a microbial efficiency at ~65% that of glucose (Frey et al. 2013). The competition of various microbial species for the limited energy and C source can affect their abundance (Fig. 5), because they differ in their rates of resource acquisition (Moore et al. 2015). Third, oxalic acid can enhance microbial access to previously physically-protected SOM by disrupting mineral-organic associations (Keiluweit et al. 2015), thereby enabling microorganisms to obtain more available substrates for the development of individual communities involved in further mineralization.

As detailed above, the underlying mechanisms of rhizosphere priming effects in regards to exudate-induced soil C losses are complex and different among the individual components of root exudates. Energetically less favourable oxalic acid accelerated the microbial mineralization of SOM and induced positive priming effects. In contrast, energetically more favourable glucose might induce apparent priming effects by increasing microbial community activities, which just assimilated easily bioavailable substrate rather than SOM as suggested by Fontaine et al. (2003). Glycine addition resulted in a suppression of microbial biomass accumulation, and thereby inhibited microbial processes and caused negative priming effects. Collectively, different exudate components delivered to the soils can stimulate microbes differing in their abundance, community composition, extracellular enzyme synthesis and the mineralization of SOM, which may be responsible for the different rhizosphere priming effects driving C losses (Fig. 1).

**Conclusions and ecological implications**

Our results demonstrated the remarkably different ecological consequences of the three common components of exudates on rhizosphere soil C losses and their underlying mechanisms: oxalic acid accelerated the loss of soil C as a result of enhanced microbial mineralization rate, whereas, glucose and glycine resulted in an increase of total soil C content in the rhizosphere soils (Fig. 2), mainly through an apparent priming effect and a negative priming effect, respectively. The magnitudes of soil C degradation in response to exudates additions were greater in the organic horizon than in the mineral horizon soil (Fig. 1, Table 3). However, the response to a specific exudate component likely followed a similar pattern in the two soils. These findings reflected the discrepant potential impacts among root exudate components on the C cycling in forest ecosystems, which should be concerned with for the purpose of better understanding soil C dynamics.

The alteration of root exudate inputs regulated by future global changes will strongly affect soil C stocks and budget in the terrestrial ecosystem. Thus, the ecological consequences of component-specialized rhizosphere processes induced by various exudate components on the soil C dynamics should be integrated into forest C cycling models to better predict soil C dynamics under global environmental changes. It must be noted that there are some limitations in the present study. For example, the present laboratory conditions cannot accurately reflect the field rhizosphere where exudate components may interactively affect the complex rhizosphere processes. As another example, the short-term incubation period of our experiment may be unable to truly reflect the long-term consequences of exudates on soil C decomposition. However, it allowed us to better understand the impacts of specific components of exudates, and to distinguish the influences of different exudates on microbially-mediated C losses.

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