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RESEARCH ARTICLE

Shifts in microbial mechanism regulate carbon priming effect under two simulated root exudate and nitrogen addition in coniferous and broad-leaved forest soils

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Abstract

Background and aims Priming effect (PE) plays a crucial role in driving soil organic carbon (SOC) decomposition and it is strongly affected by C addition types and nitrogen (N) addition. However, the understanding of the strength and microbial mechanisms of PE in response to specific root exudates (glucose and oxalic acid) and N addition remains inadequate.

Methods In this study, we carried out a 60-day incubation experiment using simulated root exudates (i.e., glucose and oxalic acid) and inorganic N in coniferous and broad-leaved forest soils to estimate their effects on PE and microbial mechanisms.

Results Oxalic acid addition resulted in positive PE through "co-metabolism" (i.e., the accelerated microbial decomposition of native SOC), which was supported by an increase in microbial biomass C (MBC), and the activities of enzyme involved in the C and N metabolism in both forest soils. In contrast, N addition significantly lowered positive PE by moderating N mining, as supported by the decreased ratios of fungi: bacteria (F: B), oxidase activities: hydrolase activities (O: H), and C: N enzyme activities, and increased CO_2 derived from root exudate per MBC. These results indicated that stoichiometric decomposition increased with the partial preferential use of the root exudate. The pattern of increased ratios of F: B, O: H, and C: N enzymes with incubation time revealed the dominance of microbial N mining.

Conclusion Collectively, our results demonstrate that shifts in driving PE from stoichiometric decomposition to microbial N mining over time predominantly depend on N availability, thereby providing insightful evidence for accurately assessing soil C dynamics for future climate change.

Keywords Co-metabolism · N availability · N mining theory · Root exudate · Priming effect · Stoichiometric decomposition theory

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Introduction

Soil is the largest carbon (C) reservoir in terrestrial ecosystems, and its C pool is higher than global vegetation and the atmosphere combined (Lehmann and Kleber 2015). The soil C pool is governed by

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the balance between the C input from plants and the output of C mainly derived from soil organic carbon (SOC) decomposition (Cotrufo et al. 2015; Castellano et al. 2015; Zhang et al. 2021). Exogenous fresh C input into soils can affect the microbial mineralization of native SOC, causing a priming effect (PE) that exerts a crucial influence on global soil C dynamic (Kuzyakov et al. 2000; Qiao et al. 2014; Kan et al. 2022). Some studies have estimated the effect of fresh C addition on PE in various ecosystems (Deng et al. 2021; Qiu et al. 2023; Siles et al. 2022; Ren et al. 2022), with 380% stimulation or 50% suppression (Cheng et al. 2014). These divergent results suggest that there exists uncertainty regarding the interactions between fresh C and microorganisms. Hence, it is important to further explore the potential microbial mechanisms of PE.

Root exudates are a labile C source that can promote microbial respiration (Finzi et al. 2015; Haichar et al. 2014). Sugar and organic acids are common compounds of root exudates and have various functions in mediating soil biogeochemical processes. The underlying mechanism can be either (1) root exudates directly change soil properties (Haichar et al. 2014) and microbial attributes (Coskun et al. 2017) or (2) root exudates like oxalic acid could interfere with or weaken the stability of mineral-organic interactions, releasing protected SOC that was previously bound to minerals (Keiluweit et al. 2015; Liao et al. 2022). These two mechanisms affect PE. Moreover, the direction and magnitude of PE would be altered with incubation time (Bernard et al. 2022; Fang et al. 2018; Zhang et al. 2021;), For instance, some studies found that PE decreased with time (Fang et al. 2018; Chao et al. 2019), while Zhang et al. (2021) suggested that PE first decreased to negative and then increased to positive effects. The main reason for these divergent results may be the altered substrate and nutrient supply, which could influence the microbial community. Nevertheless, it is unclear how different components of the exudate input interact with the microbial community over time, thus affecting PE dynamics.

Increased human activities have led to dramatic increase in nitrogen (N) deposition over the past few decades (Ackerman et al. 2019). Although N deposition has dramatically improved soil N availability throughout the world (Peñuelas et al. 2013), it can also largely mediate the intensity and direction of PE by changing soil properties and microbial attributes (Li et al. 2019; Hicks et al. 2019; Mehnaz et al. 2019). The effect of N addition on the PE remains controversial with positive (Chen et al. 2014), negative or no effect (Hicks et al. 2019; Parajuli et al. 2022; Nottingham et al. 2015). There exist two opposing microbial theories explaining the variation of PE generated by the C and N addition, namely "microbial N mining" and "microbial stoichiometry decomposition". For microbial N mining, microorganisms use labile C as a source of energy to produce more enzymes to decompose recalcitrant soil organic matter for N acquisition, likely leading to a positive PE. Accordingly, N addition is expected to reduce PE intensity by alleviating microbial nutrient deficiency. While microbial stoichiometric decomposition indicates that fast-growing microorganisms may enhance SOC decomposition by improving microbial biomass and activity when the substrate stoichiometry meets microbial physiological requirements. Consequently, a positive PE is also predominated by microbial stoichiometric decomposition theory. Previous studies have suggested that these two competing microbial mechanisms are linked and can shift over time (Fang et al. 2018). Specific microbial groups with the activity of K-strategists (e.g., fungi) mainly dominated during the microbial N mining, while the activity of r-strategists (e.g., bacteria) was important in the PE mediated by the microbial stoichiometric (Chen et al. 2014; Razanamalala et al. 2018). Nevertheless, these contrasting microbial processes have not been validated considering the relationship between PE and microbial processes under N addition over time in contrasting soils.

Here, an incubation experiment was performed to determine the influence of the simultaneous input of different root exudates and N on PE over time, and test whether PE was regulated by the shift in microbial community composition and activities in two different forest soil (i.e., coniferous forest and broad-leaved forest with *Pinus yunnanensis* and *Quercus pannosa* as the dominant species, respectively; Table S1). The soil properties were different in the two forests (e.g., pH, SOC, and C: N ratio). We hypothesized that (I) root exudate addition causes positive PE and the PE intensity under oxalic acid addition could be stronger due to liberating the mineral-associated C into soil for microbial decomposition and utilization (Keiluweit et al. 2015); (II) N addition decreases the intensity of PE in root exudate addition due to alleviating the microbial N mining, (III) "microbial stoichiometry

decomposition" dominates during the early incubation due to limited N availability (Razanamalala et al. 2018), and microbial N mining is a dominant mechanism with time after exhaustion of available N in soil (Fanin et al. 2020).

Materials and methods

Study site and experimental design

The study site was located in Yulong Snow Mountain (27°10′–27°40′N, 100°10′–100°20′E; 3200 m a.s.l) in Yunnan province in China, which is regarded as a hotspot for t biodiversity research (Niu et al. 2020). *Pinus yunnanensis* (*P. yunnanensis*) is the dominant species in e coniferous forest and *Quercus pannosa* (*Q. pannosa*) is the dominant species in broad-leaved forest. These two forest types are widely distributed in the Yunnan Province. This area has a subtropical South Asian monsoon climate, with a mean annual temperature of about 18.2 ℃, and annual precipitation of 964.7 mm. The climate was characterized by a strong dry season and wet season. Soil types were classified as Luvisols in both forests, according to the US Soil Taxonomy Series.

In November 2020, soil samples were collected in coniferous and broad-leaved forests. The two forests was 300 m apart. In each forest type, three replicates of remixed soil samples were collected from five random locations in three random plots (0–10 cm). The litter floor above the mineral soil was removed, and the soil samples were collected and mixed into one composite sample. Soil samples were immediately transferred to the laboratory and sieved (2 mm), and the visible fine root fragments were manually picked out. Finally, subsamples of soils were air-dried to measure SOC, total N, and pH. The fresh subsamples were stored in a refrigerator (< 4 °C) for further analyses such as analyzing soil available C and N (see Methods in Supporting Information). The primary soil properties are exhibited in Table S1.

Experimental design and laboratory incubation

The experimental units consisted of a 250 mL triangular flask with 30 g (dry weight equivalent) of fresh and sieved soils. All soil samples were pre-incubated at 20 °C for 14 days to reduce the influence of physical disturbance after the sampling and sieving. Before incubation, the soil moisture achieved 60% of the water-holding capacity by adding distilled water.

In order to simulate root exudate C input, six treatments were established with nine replicates using ^{13}C-labelled glucose and oxalic acid solution (representing two groups of sugars and organic acids in root exudate, respectively) after the pre-incubation: (1) soil without root exudate and N input (Control), (2) soil with N input (N), (3) soil with glucose input (G), (4) soil with inputs of glucose and N (G + N), (5) soil with oxalic acid input (O) and (6) soil with inputs of oxalic acid and N (O + N). Then, 1614.29 μg C g^{-1} and 1337.61 μg C g^{-1} of stimulated root exudate were added into coniferous soil and broad-leaved soil, respectively, which was equal to total soil microbial biomass C (MBC). This amount is similar to previous priming experiments (Bastida et al. 2013; Li et al. 2017; Chen et al. 2019). Further, 161.43 μg N g^{-1} and 133.76 μg N g^{-1} of N were added to coniferous soil and broad-leaved soil, respectively, corresponding to the C: N = 10 of the added substrate with NH_4Cl. Soil CO_2 efflux rates and the $\delta^{13}C$ were sampled on 1, 3, 5, 7, 10, 15, 20, 30 and 60 days following the beginning of treatments. To study the effect of microbial properties on PE, three soil sample replicates were destructively sampled on the 7th, 30th, and 60th days, respectively to analyze soil MBC using chloroform fumigation-extraction method (methods see supporting information) and microbial attributes (phospholipid fatty acids (PLFAs) and enzymes).

Analysis of phospholipid fatty acids (PLFAs)

PLFAs were extracted using the method depicted by Wilkinson et al. (2002). In brief, lipids were extracted twice using freeze-dried soil samples (8 g) into a buffering solvent containing a chloroform, methanol and citric acid (1:2:0.8, v/ v/ v), and using mild alkaline methanolysis to transesterify to the extracting phospholipid to FAMEs (fatty acid methyl esters). The transesterified FAMEs were determined using a gas chromatograph equipped with the MIDI Sherlock Microbial Identification System (MIDI, Inc., Newark, DE, USA) and by co-elution with standards. The PLFAs i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0was used to represent gram-positive bacteria (GP) and 16:1ω7c, 16:1ω9c, 18:1ω7c, cy17:0 and cy19:0 was used to represent gram-negative bacteria (GN) (Zhang et al. 2021). Total bacterial counts were the sum of GP and GN. The 18:1ω9c and 18:2ω6c

represent the fungi biomarkers, and 10Me18:0 was used to indicate actinomycetes (ACT) (Joergensen 2022; Lekberg et al. 2022). PLFAs were separated into four groups: GP, GN, F, and ACT.

Soil enzyme activities

The activities of eight enzymes, including six hydrolases, α-glucosidase (AG), β-glucosidase (BG), N-acetylglucosaminidase (NAG), xylanase (XYL), cellobiohydrolase (CBH), and leucine aminopeptidase (LAP), and two oxidases, polyphenol oxidase (PHO) and peroxidase (PEO), were measured using fluorometric techniques. Eight replicate wells per sample per assay were used following a modified method from Saiya-Cork et al. (2002). Soil slurries were prepared by suspending 1 g of soil in 100 ml of 50 mM sodium acetate buffer (pH5.5). In brief, the activities of AG, BG, NAG, CBH, XYL, and LAP were analyzed fluorometrically by adding 50 µL of 200 µmol L^{-1} 4-methylumbelliferyl-α-D-glucopyranoside, 4-methylumbelliferyl-β-D-glucopyranoside, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminidedehydrate, 4-methylumbelliferyl-β-Dcellobioside, 4-methylumbelliferyl-β-Dxylopyranoside and L-leucine-7-amino-4-methylcoumarin plus 200 µL of soil suspension to 96-well microtiter plates, respectively. The microplates were incubated in the dark at 20 °C for 4 h (Liao et al. 2020). Hydrolytic activity was measured fluorometrically using a microplate reader (M200 PRO; Tecan, Austria) with excitation at 365 nm and emission at 450 nm. For the oxidative enzyme, 50 µl of supernatant was added to the 200 µL of soil suspension and incubated in the dark at 20 °C for 20 h. Absorbance was measured at 460 nm using a microplate reader (M200 PRO, Tecan, Austria). The enzyme functions are listed in Table S2.

Calculations

The soil CO_2 release rate from the native SOC and root exudate during incubation was calculated as follows:

$$R_{soc} = R_{treatment} \times \left(\frac{\delta^{13}R_{treatment} - \delta^{13}C_{root}}{\delta^{13}R_{control} - \delta^{13}C_{root}} \right) \quad (1)$$

$$R_{root} = R_{treatment} - R_{SOC} \quad (2)$$

where R_{SOC} is the soil CO_2 release rate from native SOC and R_{root} is the soil CO_2 release rate derived from root exudate. $R_{treatment}$ is the total CO_2 release rate with root exudate addition, δ^{13} $R_{treatment}$ and δ^{13} $R_{control}$ is the isotope value of the total released CO_2 with root exudate addition and control, respectively. δ^{13} C_{root} is the isotope value of root exudate added to the soil samples. This equation assumed that the isotope value of root exudate and SOC are homogeneous.

During incubation, the PE caused by root exudates was calculated by comparing SOC mineralization in exudate-amended samples with control samples.

$$PE(\%) = \frac{R_{SOC} - R_{control}}{R_{control}} \times 100 \quad (3)$$

where $R_{control}$ is the CO_2 release rate in control without exudate addition.

Statistical analysis

A three-way repeated measures one-way analysis of variance (ANOVA) was used to evaluate the effects of root exudate, N addition, and forest types and their associated interactions on the total CO_2 rate, native cumulative CO_2 flux rate, exudate-derived CO_2 flux rate, relative PE, and soil microbial variables. One-way ANOVA was used to test the effect of different treatments (N and root exudate treatments) on soil enzyme activities and the main soil microbial groups at each sampling point for every forest soil sample and explore the differences between sampling times. ANOVA was performed using SPSS version 21.0. Random Forest analysis was conducted to clarify the relative contribution of enzyme activity and the main microbial groups over time in each forest soil type. Random forest analysis was performed using the R "randomForest" package in the R Statistical Environment (Version 4.1.0, R Core Team). Variable importance was indicated by the percentage increase in the mean error (% IncMSE) if a specific variable was removed, which was calculated by shuffling the values of the out-of-bag samples.

Results

SOC mineralization and PE under simulated root exudate and N addition

Native SOC mineralization and PE were significantly regulated by forest type, substrate type, N addition, and their interactions ($p < 0.05$, Table S3). Root exudate input increased native SOC decomposition, resulting in positive PE. The PE intensity was gradually decreased with time, whereas N addition significantly decreased PE in both forest soils (Figs. 1, S1, S2 and S3). The PE induced by oxalic acid was higher than that induced by glucose over the entire incubations (Fig. 1). Furthermore, Glucose addition led to a significantly higher PE in coniferous forest soil compared with broad-leaved forest soil, but the oxalic acid input did not cause significant difference in PE between the two forest types (Figs. 1 and S4).

Enzyme activities and main microbial groups under simulated root exudate and N addition

Hydrolase and oxidase activities were significantly affected by forest type, substrate type, N addition and their interactive effects (Table S4). All enzyme activities generally decreased with time, except for higher NAG activity, which was observed during mid-incubation in the coniferous forest soil (Fig. S5c). In the coniferous forest soil, glucose and oxalic acid addition significantly increased the activities of PHO and PEO across the three sampling times (Fig. S5g, h). N addition significantly decreased the activities of LAP and NAG during the first two sampling periods (Fig. S5). In the broad-leaved forest soil, root exudate addition significantly increased the activities of BG, NAG, and LAP during the first sampling period (Fig. S6b-d, f) in coniferous or broad-leaved forest. The addition of oxalic acid increased the PHO and PEO activities across the three sampling times (Fig. S6).

The ratio of C: N enzymes [(AG + BG + CBH + XYL): (NAG + LAP)] was the highest under glucose and N addition and then generally decreased with time (Fig. 2a, b). The oxidase: hydrolase ratio (O: H enzymes) [(PHO + PEO): (AG + BG + CBH + XYL + NAG + LAP)] was significantly higher with oxalic acid input in coniferous forest soil (Fig. 2c), whereas it was significantly higher

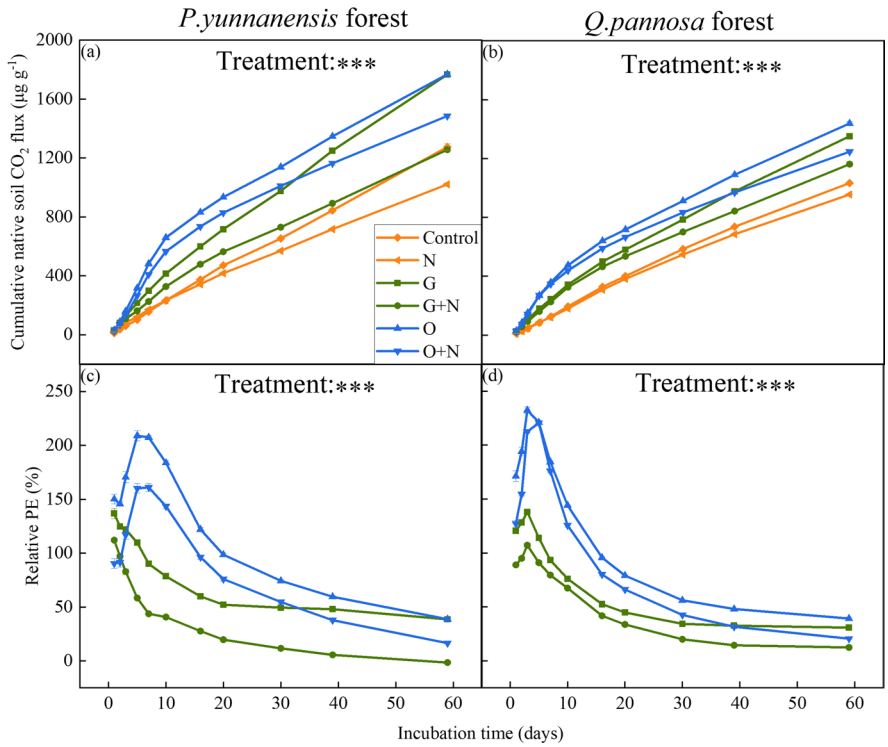

Fig. 1 Priming effect (PE) of root exudate and N addition on cumulative native soil CO_2 flux and relative PE in the coniferous forest (**a** and **c**) and broad-leaved forest (**b** and **d**) soils during the 60-day incubation. Values are means with standard error ($n = 3$). Six treatments included that (1) soil without root exudate and N additions (Control), (2) soil with N addition (N), (3) soil with glucose addition (G), (4) soil with combined additions of glucose and N (G + N), (5) soil with oxalic acid addition (O) and (6) soil with combined additions of oxalic acid and N (O + N)

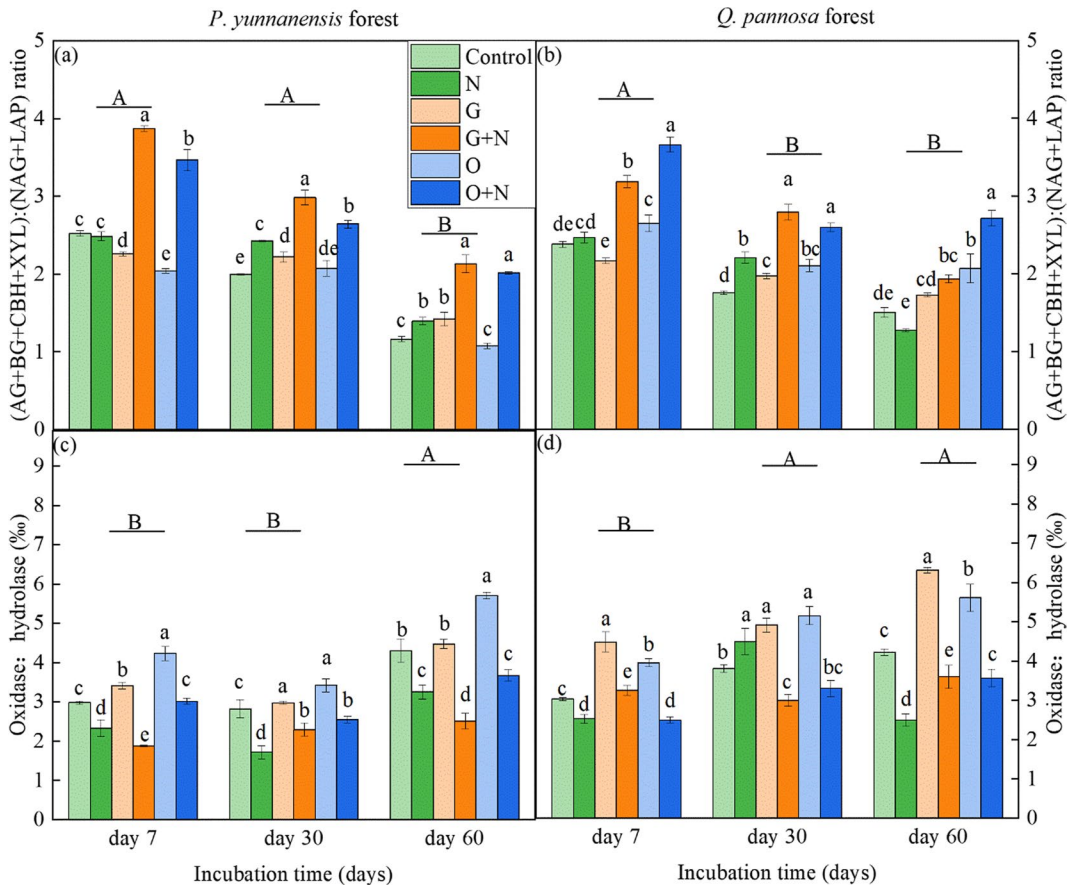

Fig. 2 Effect of root exudate and N addition on soil C:N enzyme activity ratio [(AG + BG + CBH + XYL): (NAG + LAP)]and oxidase: hydrolase activity ratio [(PHO + PO):(AG + BG + CBH + XYL + NAG + LAP)] during three sampling times in the coniferous (**a** and **c**) and broad-leaved (**b** and **d**) forest soils. AG (α-Glucosidase); BG (β-Glucosidase); NAG (N-acetyl-β-glucoasminidase); CBH (Cellobiohydrolase); XYL (Xylosidase); LAP (Leucine amino peptidase); PHO (Polyphenol oxidase) and PEO (Peroxidase). Different capital letters displayed at the top of periods represent significant difference among periods under treatments. Different lowercase letters above bars represent significant differences among treatments within periods. Significance levels are set at $p < 0.05$. See Fig. 2 for abbreviations

with glucose addition in broad-leaved forest soil (Fig. 2b, d). The oxidase-to-hydrolase ratio gradually increased over time in both forest soil types (Fig. 2d).

The microbial community composition groups were also affected by substrate type, N addition, and sampling time (Table S4). The GP: GN and F: B ratios increased with time, and N addition significantly decreased the F: B ratio in both the glucose and oxalic acid treatments across the three sampling times in both forest soils (Figs. 3 and 4).

Drivers of PE with incubation time

Random forest analysis indicated that the importance of oxidase: hydrolase, C: N enzyme activities, PEO activity, and N availability to PE increased with the extending of incubation time, whereas the importance of AG, ACT, and NAG activities to PE decreased with incubation time in coniferous forest soil (Fig. S7a). For broad-leaved forest soil, the importance of the oxidase: hydrolase ratio, PHO,

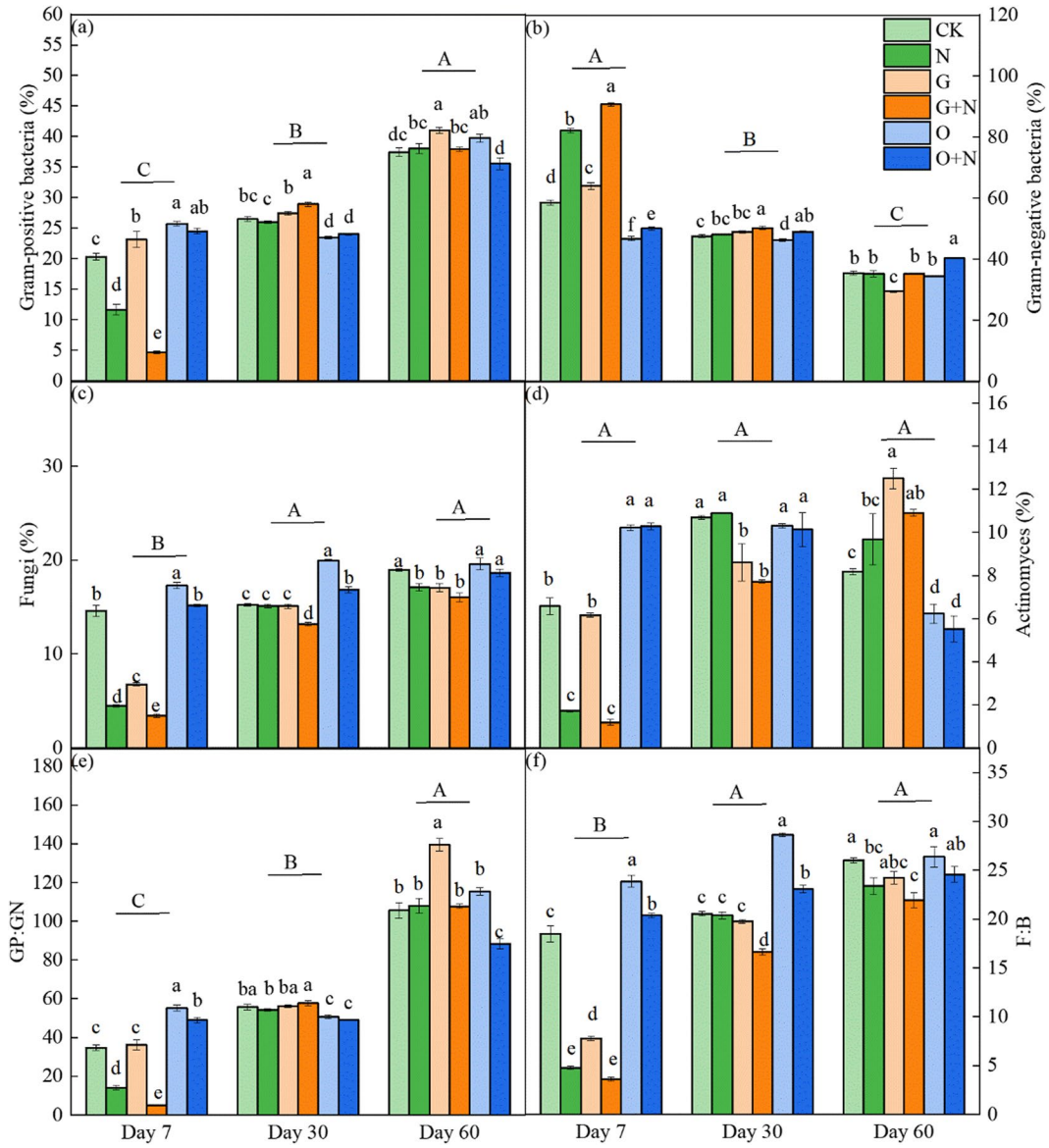

Fig. 3 Effect of root exudate and N addition on main soil microbial groups during three sampling times in the coniferous forest soil including (**a**) gram-positive bacteria (GP); **b** gram-negative bacteria (GN); **c** Fungi (F); **d** Actinomyces (ACT); **e** the ratio of GP and GN; **f** the ratio of fungi and bacteria. Values are means with standard error ($n = 3$). Different capital letters at the top of periods represent significant difference among periods under treatments. Different lowercase letters above bars represent significant differences among treatments within periods. Significance levels are set at $p < 0.05$

C: N enzyme activities, and N availability to PE increased with the extending of incubation time, whereas the importance of PEO, AG, and BG activities and the GP: GN ratio on PE decreased with incubation time (Fig. S7b).

Discussion

Our results showed that root exudate input had a positive priming effect by increasing the SOC decomposition rate in both forest soils (Fig. 1 and S1-S3). The

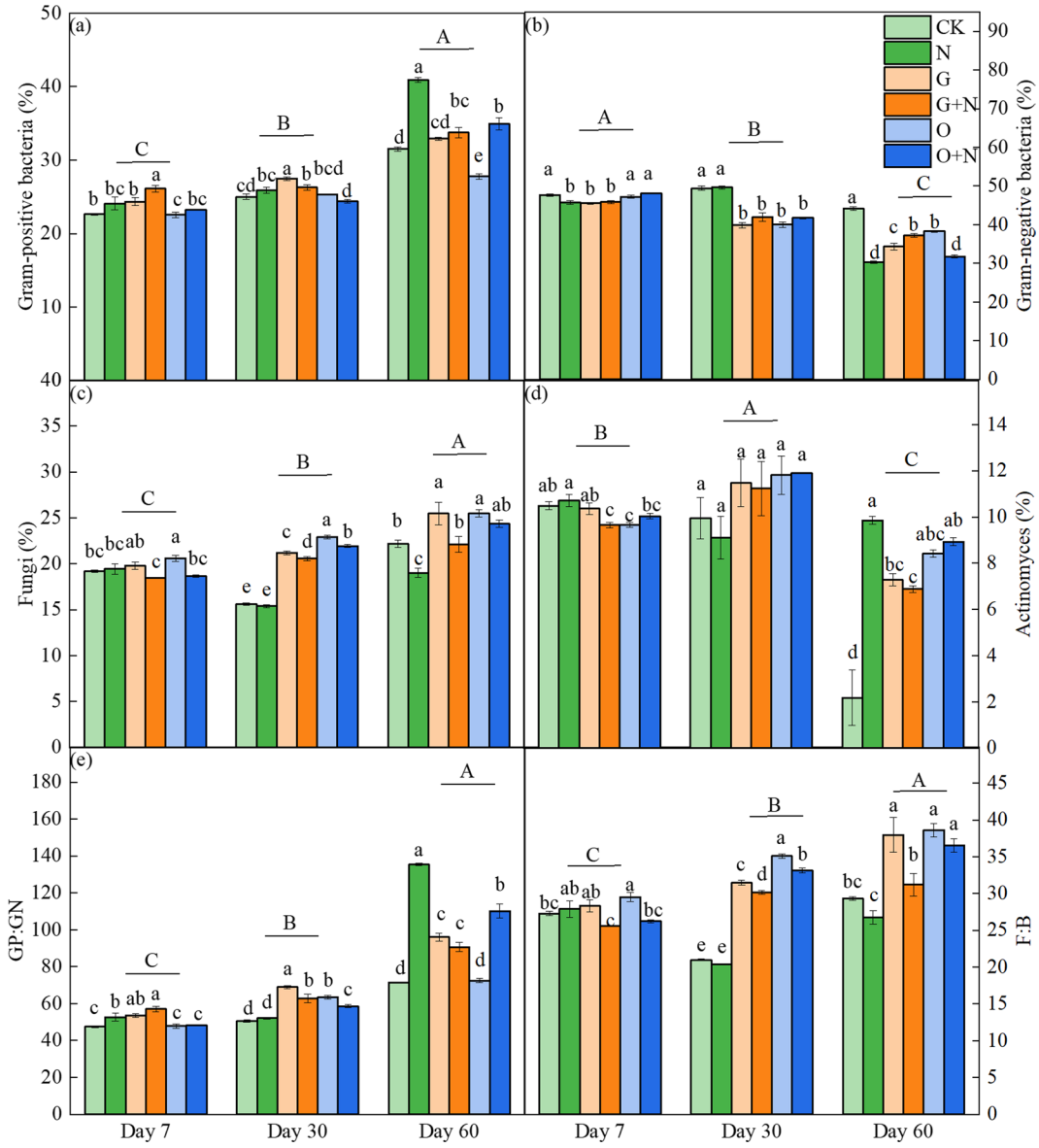

Fig. 4 Effect of root exudate and N addition on main soil microbial groups during three sampling times in the broad-leaved forest soil including (**a**) gram-positive bacteria (GP); **b** gram-negative bacteria (GN); **c** Fungi; **d** Actinomyces; **e** the ratio of GP and GN; **f** the ratio of fungi and bacteria. Values are means with standard error ($n=3$). Different capital letters displayed at the top of periods represent significant difference among periods under treatments. Different lowercase letters above bars represent significant differences among treatments within periods. Significance levels are set at $p<0.05$. See Fig. 5 for abbreviations

findings were similar with previous studies reporting a positive PE in response to labile C input from different forest types (Chao et al. 2019; Lyu et al. 2018; Sullivan and Hart 2013; Wild et al. 2014). The observed positive priming effect under root exudate input could be attributed to microbial co-metabolism (Fontaine et al. 2003; Perveen et al. 2019), namely, the accelerated microbial decomposition of native SOC using the added root exudate as energy compound (Fontaine et al. 2003), as supported by the increase in MBC under root exudate addition compared with the control (Figs. S5, S6, and S7). The

positive relationship between PE and enzyme activity also supports this observation (Fig. S9).

We found that oxalic acid input led to a larger PE than did glucose input in both forest soils (Fig. 1), suggesting that the type of root exudate was one of the major drivers of the priming effect. These results are consistent with our first hypothesis. Several possible reasons may be responsible for this result. First, oxalic acid can disrupt physically protected mineral-organic associations (Wang et al. 2021), and increase available C pools for soil microorganisms and their enzyme activities (Clarholm et al. 2015). This process strongly promotes microbial SOM decomposition, ultimately leading to a stronger priming effect. Partially consistent with this hypothesis, we observed higher C availability with the input of oxalic acid at the beginning of the incubation in broad-leaved forest (Fig. S10). Second, microorganisms prefer to utilize glucose with a lower degree of reduction of C for incorporation into microbial biomass relative to oxalic acid (Manzoni et al. 2012; Mehnaz et al. 2019), implying that microorganisms have higher C-use efficiency under glucose addition than under oxalic acid addition, possibly lowering the priming effect under glucose addition. The higher substrate-derived MBC confirmed a lower priming effect owing to higher C utilization with glucose addition (Fig. S8). Third, oxalic acid addition stimulated fungal growth (Figs. 3 and 4), and fungi efficiently degraded recalcitrant SOC with higher oxidase activity (Fig. S9; Fontaine et al. 2003, 2011). Consequently, higher fungal abundance with oxalic acid addition could enhance the priming effect. These results contradict those of a previous study showing that oxalic acid addition caused negative or no PE owing to the structural rearrangement of recalcitrant materials (Hamer and Marschner 2005). Collectively, our results indicate that oxalic acid addition promotes SOC mineralization jointly by abiotic and biotic processes, emphasizing that the two exudate compounds affect soil C decomposition through various regulation (Wang et al. 2021).

Consistent with our second hypothesis, we also observed that N addition lowered the positive priming effect regardless of the root exudate components (Fig. 1), which could be attributed to a reduction in the "microbial N-mining" theory (Dimassi et al. 2014; Fontaine et al. 2011; Liao et al. 2020). This was also observed by Perveen et al. (2019) and Feng et al. (2021). This may explain the decreased LAP and NAG activities under N addition, especially during the first two incubation periods (Figs. S5c, f and S6c, f).

N addition reduced microbial N mining and lower PE mediated by microbial stoichiometric decomposition (Fig. 5). The abundance of fungi and oxidase activity decreased, while bacterial abundance increased under N addition in both forest soils (Figs. 2, 3, 4, and S5-6). The microbial stoichiometric theory has been used to explain the enhanced PE in response to N addition (Meyer et al. 2018; Zhang et al. 2021), but the PE decreased in both forest soils

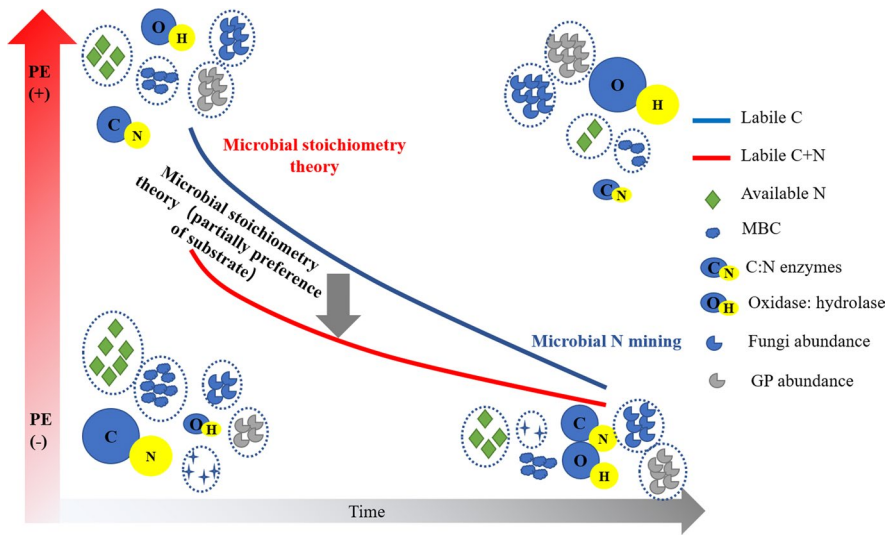

Fig. 5 A conceptual overview of the priming effect induced by root exudate and N additions over 60-day incubation. The red and blue solid lines represent the root exudate with and without N addition, respectively

when both root exudations were supplemented with N (Figs. 1, S1, and S2). The decrease in PE was likely due to the preferential use of root exudates, as indicated by the higher CO_2 derived from root exudates per MBC under N addition in both forest soils (Fig. S11). The results emphasized the negative effect of PE to N addition due to the stoichiometric decomposition with preferential use of substrate.

The difference in the priming effect was higher under root exudates with N addition than with root exudates alone (Figs. 1 and S2), likely due to the dominance of the microbial N mining mechanism without N addition, which increased with incubation time because of the rapid depletion of N (Razanamalala et al. 2018), leading to a higher C: N imbalance for microorganisms (Chen et al. 2018). Under N-depleted conditions, microorganisms may stimulate SOM mineralization by secret oxidase to decompose recalcitrant organic matter and obtain N source s (i.e., microbial N mining) (Fanin et al. 2020). This may explain the increase in the oxidative hydrolase enzyme activity ratio and decrease in the C: N enzyme activity ratio with incubation time. Moreover, the increase in fungal abundance over time irrespective of N addition in both forest soils (Figs. 3 and 4) may further support the role of K-strategists in microbial N mining theory over time. Thus, our results indicated a shift in microbial mechanisms with enhanced microbial N mining over time, regardless of N addition in both forest soils, corresponding to our third hypothesis.

Additionally, we found that the priming effect induced by glucose was greater in the coniferous forest soil than in the broad-leaved forest soil, whereas there was no significant difference under oxalic acid addition over the entire incubation period between the two forest soils (Figs. 1 and S3). These divergent results are likely due to the different SOC recalcitrance between the two forest soils, with higher aromatic compounds in fresh leaves and higher soil DOC in the coniferous forest soil (Fig. S12), leading to higher microbial C limitation and a larger proportion of microbial dormancy (Barré et al. 2016). Consequently, the percentage of microorganisms activated by root exudate input is higher in coniferous forest soil than in broad-leaved soil, stimulating greater SOC decomposition (Blagodatskaya and Kuzyakov 2013). When oxalic acid is added, the released C induced by oxalic acid may also be recalcitrant to microbial utilization because of the higher aromatic compound content, which can balance the higher priming effect induced by stronger C limitation in coniferous forest soil (Bastida et al. 2019; Guo et al. 2016; Keiluweit et al. 2015).

Conclusions

In agreement with the suggested hypothesis, our results suggested that two root exudates caused positive priming effect through "co-metabolism" with a higher priming effect under oxalic acid than that under glucose. Furthermore, N addition significantly decreased the magnitude of the positive priming effect, likely because microbial N mining was reduced and microbial stoichiometry decomposition was enhanced with partial preferential use of root exudate, which was supported by the decrease in fungi to bacteria (F: B), C to N enzyme activities (C: N enzymes), and oxidase: hydrolase ratios, as well as higher root exudate-derived CO_2 flux per microbial biomass C under N addition. The increased ratios of C: N enzymes, F: B, and oxidase: hydrolase with time suggest that both stoichiometric decomposition and microbial N mining could influence the priming effect, with an increasing contribution of the microbial N mining mechanism across treatments with time (Fig. 5).

Our results emphasize the shift in microbial mechanisms from stoichiometric decomposition to microbial N mining with time likely due to the declined C and N availability, thereby providing insightful evidence for accurately evaluating the soil C dynamics for future climate change. The PE is a complex process in which microbial N mining or stoichiometric decomposition theory cannot solely explain the variation of PE. Thus, combined the two theories could provide a critical clue to further explore the PE mechanisms (Feng et al. 2021). In the future, more effort is necessary to quantify the broad patterns of microbial mechanisms underlying the priming effect and reveal its response to N deposition. Nevertheless, more precise measurement of microbial communities is also urgent to further elucidate the microbial C utilization processes for a better knowledge gain of soil-C dynamics under global change.

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Author contributions X.L.C and C.L conceived the ideas and designed methodology; C.L and Y.B collected the data; C.L analyzed the data; X.L.C and C.L led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing interest The authors declare no competing of interest.

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