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Research

Rhizosphere priming effects on soil carbon and nitrogen dynamics among tree species with and without intraspecific competition

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Summary

- Rhizosphere priming effects (RPEs) play a central role in modifying soil organic matter mineralization. However, effects of tree species and intraspecific competition on RPEs are poorly understood.
- We investigated RPEs of three tree species (larch, ash and Chinese fir) and the impact of intraspecific competition of these species on the RPE by growing them at two planting densities for 140 d. We determined the RPE on soil organic carbon (C) decomposition, gross and net nitrogen (N) mineralization and net plant N acquisition.
- Differences in the RPE among species were associated with differences in plant biomass. Gross N mineralization and net plant N acquisition increased, but net N mineralization decreased, as the RPE on soil organic C decomposition increased. Intraspecific competition reduced the RPE on soil organic C decomposition, gross and net N mineralization, and net plant N acquisition, especially for ash and Chinese fir.
- Microbial N mining may explain the overall positive RPEs across species, whereas intensified plant–microbe competition for N may have reduced the RPE with intraspecific competition. Overall, the species-specific effects of tree species play an important role in modulating the magnitude and mechanisms of RPEs and the intraspecific competition on soil C and N dynamics.

Introduction

Forest ecosystems contain *c.* 45% of the global terrestrial carbon (C), with *c.* 383 ± 30 Pg organic C stored in soil organic matter (SOM) to 1-m depth (Pan *et al.*, 2011). This SOM in forest ecosystems plays a critical role in mitigating the rise in global atmospheric CO_2 concentration and stabilizing the climate system (Cleveland *et al.*, 2013). There is increased recognition that rhizosphere processes can significantly influence SOM decomposition and nutrient (especially nitrogen, N) cycling (Phillips *et al.*, 2011; Zhu *et al.*, 2014; Finzi *et al.*, 2015), but our understanding of the effects of rhizosphere interactions on SOM decomposition and plant N availability among tree species is rudimentary.

The rhizosphere priming effect (RPE) is the acceleration or retardation of SOM decomposition by living roots and associated rhizosphere organisms (Kuzyakov, 2002; Cheng & Kuzyakov, 2005), and is one of the most important components of rhizosphere interactions (Finzi *et al.*, 2015). Compared to rootless soil, recent studies have shown that SOM decomposition with living roots can increase by up to 380% (positive RPE), or reduce by up to 50% (negative RPE) (Cheng *et al.*, 2014). Given that the RPE plays a quantitatively pivotal role in regulating SOM turnover and nutrient cycling (Cheng & Kuzyakov, 2005) and that inclusion of the RPE can substantially improve the performance of ecosystem and global biogeochemical models (Cheng *et al.*, 2014; Sulman *et al.*, 2014), it becomes critical to understand the driving factors and mechanisms behind RPEs.

Previous studies under artificial environmental conditions have shown that the magnitude and direction of RPEs vary significantly with plant species and soil variables (summarized by Cheng *et al.*, 2014). Using ^{13}C-labeling approaches, it was shown that the direction and magnitude of the RPE depend on soil type and N status (Fontaine *et al.*, 2011; Kumar *et al.*, 2016; Lloyd *et al.*, 2016), but also on plant species and associated traits, such as rhizosphere acidification (Wang *et al.*, 2016), fine root morphology (Pausch *et al.*, 2016) and root exudation (Dijkstra & Cheng, 2007; Bengtson *et al.*, 2012; Zhu *et al.*, 2014). Nevertheless, effects of tree species on RPEs remain unclear, even though a

recent meta-analysis showed that trees caused the highest RPE compared to other functional types such as grasses and crops (Huo *et al.*, 2017). Furthermore, compared to C decomposition, little attention has been paid to RPEs on N mineralization and plant N uptake (Frank & Groffman, 2009; Kuzyakov, 2010; Cheng *et al.*, 2014).

The influence of plant intraspecific competition on RPE has not been investigated, even though intraspecific competition is ubiquitous and plays an important role in root interactions (Schenk, 2006). In the few studies where interspecific interactions on RPE were investigated it was shown that competition for soil mineral N between plants and microbes could be intensified due to complementarity and selection effects on plant N uptake thereby reducing the RPE (Dijkstra *et al.*, 2010; Pausch *et al.*, 2013). Furthermore, when plants share the same soil (regardless if they are of different or the same species), this can alter their root biomass and distribution, and modify their root physiology, resulting in greater depletion of soil nutrients (Beyer *et al.*, 2013; Donnelly *et al.*, 2016). Thus, intraspecific competition (especially for nutrients) may also modify the plant–microbe–soil interactions, and this modification may also be species-specific. Therefore, it is imperative to gauge the extent to which such intraspecific interactions regulate RPEs on SOM decomposition and N dynamics.

The underlying mechanisms of RPEs are poorly understood (Cheng *et al.*, 2014). An enhanced RPE can be attributed to increases in microbial activity and enzyme production induced by root exudates to accelerate the availability of N stored in SOM ('microbial N mining' hypothesis) (Craine *et al.*, 2007; Fontaine *et al.*, 2011; Dijkstra *et al.*, 2013). A depressed RPE, especially with low nutrient concentrations, is usually ascribed to the 'nutrient competition' hypothesis (Kuzyakov, 2002; Cheng & Kuzyakov, 2005), where depletion of mineral nutrients due to plant uptake decreases microbial growth and metabolism, thereby reducing SOM decomposition. Therefore, the magnitude and direction of RPEs depend on complex interactions of substrate availability, such as root exudates, soil mineral nutrients, and microbial growth and activity. However, the conditions under which these mechanisms dominate and the extent to which such factors influence RPEs have not been clearly assessed.

Here, three commercially important tree species, larch (*Larix kaempferi*), ash (*Fraxinus mandshurica*) and Chinese fir (*Cunninghamia lanceolata*) from different families were planted in a C_4 soil (which was under C_4-vegetation for 23 yr). These species differ greatly in life form, growth rate, mycorrhizal association and root traits (Supporting Information Tables S1, S2), and have different effects on soil C and N cycling (Wang *et al.*, 2014, 2017). We calculated the RPE on soil C and N mineralization using a ^{13}C natural abundance approach (Cheng *et al.*, 2003) and a ^{15}N pool dilution method (Wu *et al.*, 2012), respectively. We also determined net N mineralization based on changes between final and initial soil inorganic N and plant biomass N pools (Zhu & Cheng, 2012). Microbial biomass C and potential extracellular enzyme activities were measured to investigate changes in microbial activities. We hypothesized that all three species showed positive RPE on soil C and N mineralization due to the microbial mining of N from SOM. Moreover, intraspecific competition reduced the RPE of these three species because of the intensified competition of N between plants and microbes. Both the intensity of RPE and its response to intraspecific competition may differ among species, likely caused by species-specific plant traits such as growth rate, N demand and exudation rate.

Materials and Methods

Experimental setup

The carbon-13 (^{13}C) natural tracer approach was used to partition soil-derived CO_2–C (C_4–C) from root-derived CO_2–C (C_3–C) by planting C_3 plants in C_4 soils (Cheng *et al.*, 2003). The soil was developed under long-term continuous cropping of a C_4 plant (maize, > 23 yr). The surface soil (0–20 cm, Mollisol) was collected, sieved and homogenized through a 5-mm screen, then air-dried. It contained 1.84% organic C, 0.14% nitrogen (N), 43% sand, 22% silt, and 35% clay, and had a pH of 6.8. The mean $\delta^{13}C$ value of respired CO_2 from the C_4 soil was *c.* −17‰, whereas the mean $\delta^{13}C$ value of CO_2 from C_3 root respiration was < −27‰. Therefore, the > 10‰ difference in $\delta^{13}C$ values between soil and root respiration worked well to separate the soil- from the root-derived CO_2 (Cheng *et al.*, 2003).

The experiment used three species (larch (*Larix kaempferi* (Lamb.) Carriere), ash (*Fraxinus mandshurica* Rupr.) and Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook.)), two planting densities with an unplanted control and one destructive sampling time (140 d after planting). There were 27 pots in total, with four replicates of each treatment (except for larch single-seedling treatment where there were only three replicates, because one of the seedlings died during the experiment). Notably, rhizosphere effects of these tree seedlings in a non-native C_4 soil in pots may be different than those of mature trees in their native C_3 soils in the field. However, we aimed to unravel mechanisms for rhizosphere priming effect (RPE) and its response to intraspecific competition, rather than to extrapolate the results to native soils and field conditions. A summary of similar studies on RPE for tree species is provided in Table S3.

The polyvinyl chloride (PVC) pots (diameter 20 cm, height 40 cm) were closed at the bottom, equipped with a plastic tube inside. One end of the tube bound with a nylon bag filled with 100 g washed sand was placed at the bottom of each pot (for aeration and CO_2 trapping), whereas the other end was kept outside of the pot. Then, 10 kg air-dried soil (equal to 9.7 kg oven-dried soil) was packed into each pot. We selected 2-yr-old seedlings based on similarity in size (height 70 cm, stem diameter at ground level 1.5 cm). After carefully washing off the adhering soil without damaging the root system, seedlings were transplanted to the pots in May 2015. We also grew other seedlings in the field for measuring the $\delta^{13}C$ fractionation of root respiration later (see 'Calculations'). Four extra seedlings of each species were weighed and analyzed for dry shoot and root biomass, and their N content at the time of planting (Table S1). All pots were added with 58–63 g of fresh native forest soil (equal to 0.5% of C_4 soil) to ensure adequate specific mycorrhizal infection of each seedling. The pots were under a rainout shelter (length 24 m, width 8 m and height

1.6 m with a transparent plastic sheet on the top letting in most of the sunlight) located at the National Field Research Station of Shenyang Agroecosystems in Shenyang, Liaoning (41°31′N, 123°24′E). The shelter had only minor effects on air temperature and humidity (Su *et al.*, 2017).

The seedlings were grown for 140 d (approximately a whole growing season) before harvesting in the middle of September 2015. During seedling growth soil moisture in each pot was maintained at 80% water holding capacity (24% gravimetric soil moisture content) by watering gently with tap water every 2 d based on weight loss of the pots. To avoid anaerobic conditions, the tube inside each pot was connected to an aquarium air pump to force fresh ambient air into the soil for 1 h every 6 h every day controlled by a timer.

Soil CO_2 efflux

We measured soil respiration of each pot by a closed-circulation CO_2 trapping system (Cheng *et al.*, 2003) before harvesting. Briefly, we sealed each pot with nontoxic silicone rubber after placing two half-moon PVC covers on top of the soil inside the pots surrounding the stems of the seedlings and with the tube for air circulation protruding through the seal. The CO_2 inside the pots was then scrubbed by circulating the isolated air through a soda lime column (3 cm diameter, 100 cm length) for 2 h. Then CO_2 produced in each pot during a 24 h-period was trapped in 300 ml of 0.5 M NaOH solution. Meanwhile, extra pots filled with acid-washed sand and planted with one seedling of each species (four replicates) dug up from the field were sealed and CO_2 was trapped in the same way. These pots were used to calculate the $\delta^{13}C$ fractionation of root respiration (see 'Calculations'). We further included four blanks to correct for background carbonate concentrations in the NaOH stock solution. During trapping, the air was circulated for 40 min at a 4-h interval by a timer. An aliquot of each NaOH solution was analyzed for total inorganic C using a TOC analyzer (Vario TOC cube, Elementar Analysis system GmbH, Langenselbold, Germany) and another aliquot for $\delta^{13}C$ using cavity ring-down spectroscopy (CRDS) with Automate Module (Picarro G2131-i Analyzer, Picarro Inc., Santa Clara, CA, USA).

Harvesting and measurements after harvest

After CO_2 trapping, the pots were destructively harvested. The seedling shoots were cut off at the soil surface, and roots were separated and hand-picked. At the time of harvesting we observed minor root bounding in the ash double-seedling pots, but not in the other two species (Fig. S1; Table 1). Shoots and roots were washed, oven-dried at 65°C for 48 h, weighed, ground in a ball mill and measured for total C and N using an elemental analyzer (vario MACRO cube, Elementar) and for $\delta^{13}C$ using the Picarro G2131-i Analyzer. Soil mineral N ($NH_4^+ + NO_3^-$) was determined by extracting 30 g of fresh soil with 60 ml 2 M KCl solution immediately after sampling, and soil moisture was measured by oven-drying soil at 105°C for 48 h. The extracts were analyzed for NH_4^+ and NO_3^- on a Continuous Flow Analyzer (AA3, Bran+Luebbe, Norderstedt, Germany).

Table 1 Plant shoot and root biomass, shoot : root ratio, net plant nitrogen (N) acquisition, growth rate and competition intensity index (CII) at the end of the experiment

	Plant biomass (g per plant)			Net N acquisition (mg N kg^{-1} soil)				Growth rate (g d^{-1})	CII
Treatment	Shoot	Root	Shoot : root	Shoot N	Root N	Shoot : root	Total N		
Single-seedling									
Larch	$13.1 \pm 2.0^{a,b}$	5.22 ± 1.0^{a}	$2.64 \pm 0.5^{a,b,c}$	8.45 ± 3.1^{a}	3.78 ± 1.4^{a}	2.19 ± 0.3^{c}	12.2 ± 4.5^{a}	$0.07 \pm 0.0^{a,b}$	
Ash	37.2 ± 3.8^{c}	34.0 ± 3.6^{c}	1.09 ± 0.0^{a}	$28.6 \pm 4.9^{b,c}$	$27.3 \pm 3.9^{a,b,c}$	$1.04 \pm 0.1^{a,b}$	$55.9 \pm 3.2^{b,c}$	$0.28 \pm 0.1^{c,d}$	
Chinese fir	57.6 ± 5.4^{d}	17.5 ± 1.8^{b}	3.32 ± 0.1^{c}	57.5 ± 2.8^{d}	17.2 ± 0.7^{b}	3.37 ± 0.3^{d}	74.7 ± 2.4^{c}	0.43 ± 0.1^{d}	
Double-seedling									
Larch	10.8 ± 1.2^{a}	4.77 ± 0.3^{a}	$2.32 \pm 0.4^{a,b,c}$	9.57 ± 3.2^{a}	6.26 ± 0.9^{a}	$1.43 \pm 0.4^{a,b,c}$	15.8 ± 4.0^{a}	0.05 ± 0.0^{a}	0.17 ± 0.1
Ash	$26.3 \pm 1.2^{b,c}$	32.1 ± 0.6^{c}	0.82 ± 0.1^{a}	$14.3 \pm 2.6^{a,b}$	$31.4 \pm 2.1^{b,c}$	0.46 ± 0.1^{a}	45.7 ± 3.2^{b}	$0.20 \pm 0.0^{b,c}$	$0.29 \pm 0.1^{*}$
Chinese fir	29.2 ± 0.4^{c}	16.7 ± 0.2^{b}	1.75 ± 0.0^{b}	39.1 ± 1.6^{c}	24.9 ± 1.0^{c}	1.57 ± 0.1^{bc}	63.9 ± 2.3^{bc}	0.24 ± 0.0^{c}	$0.49 \pm 0.0^{*}$
ANOVA *P*-values									
Species	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.0001	<0.001	
Density	<0.001	0.60	0.002	0.001	0.01	<0.001	0.15	<0.001	
Species × Density	0.06	0.99	0.02	0.02	0.46	0.03	0.26	0.02	

For each column (except for CII), significant differences among six planted treatments are denoted by different letters (*post hoc* Tukey–Kramer honest significant difference (HSD) test, $P < 0.05$). Net plant N acquisition equals plant N content at harvest subtracted by initial plant N content when planted. The average shoot biomass of two seedlings is used for calculating CII. CII values significantly larger than zero in the double-seedling treatments are presented as * (two-tailed *t*-test, $P < 0.05$). Values represent mean ± SE; $n = 8$ for the double-seedling treatments; $n = 4$ for the single-seedling treatments except for larch single-seedling treatment where $n = 3$.

Gross N mineralization rate (GNM) was measured using the ^{15}N pool dilution method described by Wu *et al.* (2012) with some modifications. Briefly, 60 g of fresh soil was homogeneously labeled immediately after sieving with 3 ml $(^{15}NH_4)_2SO_4$ solution at 30 atom% ^{15}N enrichment (the total $NH4^+$-N content was increased by approximately 1 mg N kg^{-1} soil). Then each soil was divided in half, incubated at 25°C and extracted with 60 ml of 2 M KCl at 18 h and 42 h after labeling. Ammonium concentration in the extracts was measured using the Continuous Flow Analyzer. Then, NH_4^+ in the extracts was collected using diffusion traps (Dannenmann *et al.*, 2009), and the ^{15}N atom% determined by an Isotope Ratio Mass Spectrometer (MAT 253; Thermo Fisher, Bremen, Germany) coupled with an Elemental Analyzer (Flash EA 1112; Thermo Fisher). Gross ammonification was calculated following the equations in (Kirkham & Bartholomew, 1954).

Microbial biomass C (MBC) was measured using the chloroform fumigation-extraction method (Vance *et al.*, 1987). One 30 g subsample was fumigated by chloroform in the dark for 24 h and then extracted with 60 ml 0.5 M K_2SO_4 solution, and another 30 g subsample was extracted with 60 ml 0.5 M K_2SO_4 solution without fumigation. The extracts were measured for total organic C using the vario TOC cube (Elementar). MBC was then calculated as the difference between fumigated and non-fumigated extracts, adjusted by a proportionality coefficient ($k_{EC} = 0.45$).

We determined potential extracellular enzyme activities (EEAs) of four hydrolytic and two oxidative enzymes based on their ability to decompose organic C or depolymerize organic N, according to a fluorometric method modified from (Saiya-Cork *et al.*, 2002). Briefly, β-cellobiohydrolase (CB), β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG) and leucin aminopeptidase (LAP) were determined using 4-methylumbelliferyl β-D-cellobioside, 4-methylumbelliferyl β-D-glucopyranoside, 4-methylumbelliferyl N-acetyl-β-D-glucosaminide and L-leucine-7-amio-4-methylcoumarin hydrochloride as substrates, respectively. Phenol oxidase (PO) and peroxidase (PER) activities for recalcitrant organic C and N degradation were assayed using L-3,4-dihydroxyphenylalanine substrate. The soil suspension was dispersed by thoroughly mixing 1 g fresh soil with 125 ml buffer (50 mM Tris, pH = 7.0). Then 50-μl aliquots were dispensed in 96-well microplates (Brand pureGrade, black), followed by appropriate standards, homogenates and substrates. After placing microplates in the dark at 25°C for 4 h (four hydrolytic enzymes) or 24 h (two oxidative enzymes), we assayed fluorescence for four hydrolytic enzymes on a microplate reader (BioTek Synergy HT MultiMode; BioTek Instrument, Winooski, VT, USA) with 365 nm excitation and 450 nm emission filters, and absorbance for two oxidative enzymes with a 460 nm filter. Enzyme activities were calculated as nmol g^{-1} soil h^{-1} for the six enzymes.

Calculations

We calculated the CO_2 derived from SOM decomposition ($C_{soil\text{-}derived}$, mg C kg^{-1} soil d^{-1}) using a two-source mixing model:

$$C_{soil-derived} = C_{total}(\delta^{13}C_{root-derived} - \delta^{13}C_{total})/(\delta^{13}C_{root-derived} - \delta^{13}C_{soil-derived}) \quad (1)$$

$$C_{root-derived} = C_{total} - C_{soil-derived} \quad (2)$$

(C_{total}, total soil respiration; $\delta^{13}C_{total}$ and $\delta^{13}C_{soil\text{-}derived}$, measured $\delta^{13}C$ values of soil respiration in the planted and unplanted soil (‰), respectively; $C_{root\text{-}derived}$, root-derived CO_2 from the planted pots (mg C kg^{-1} soil d^{-1}); $\delta^{13}C_{root\text{-}derived}$, $\delta^{13}C$ value of root respiration (‰) derived from $\delta^{13}C$ values of root biomass).

We calculated the $\delta^{13}C$ fractionation (f, ‰) as the difference between $\delta^{13}C$ in root respiration and in root biomass of the plants transplanted into the acid-washed sand. The mean f values for larch, ash and Chinese fir were −1.7‰, −2.0‰ and −1.3‰, respectively. We assumed that the effect of intraspecific competition on f values was negligible according to the results of Beyer *et al.* (2013). We therefore determined the $\delta^{13}C_{root\text{-}derived}$ for single- and double-seedling treatments as the $\delta^{13}C$ value of root biomass plus f of each species.

The observed primed C was calculated as the difference in $C_{soil\text{-}derived}$ between the planted and unplanted treatment (mg C kg^{-1} soil d^{-1}):

$$\text{Primed C} = C_{soil-derived\ (planted)} - C_{soil-derived\ (unplanted)} \quad (3)$$

We calculated net plant N acquisition (N_{acq}, mg N $kg\ soil^{-1}$) as the difference in total N content of seedlings per unit of soil DW between planting and harvest, and the net change in soil mineral N ($N_{min\ (end\text{-}start)}$) as the difference in total soil mineral N between planting and harvest. We then calculated the rate of N made available to plants (N_{ava}, mg N kg^{-1} soil d^{-1}) as the difference in net plant N acquisition and net change in soil mineral N, divided by time (Dijkstra *et al.*, 2009):

$$N_{ava} = [N_{acq} + N_{min\ (end-start)}]/\text{time} \quad (4)$$

Assuming that gaseous N loss was relatively small (while no N leaching occurred), N_{ava} can be considered as the net N mineralization rate. We also calculated 'excess net N mineralization' and 'excess gross N mineralization' as the difference in N_{ava} and gross N mineralization between planted treatments and unplanted control, respectively, where excess gross N mineralization can be considered to be primed N (Dijkstra *et al.*, 2009).

We determined the effect of intraspecific competition on primed C, primed N, root-derived CO_2, excess net N mineralization, N_{acq}, MBC, EEAs and soil mineral N by comparing the observed values (Observed-d_i) in the double-seedling treatments with expected values (Expected-d_i) based on values observed in single-seedling treatments adjusted for root biomass measured in single- and double-seedling treatments (Dijkstra *et al.*, 2010; Pausch *et al.*, 2013). Expected values were calculated as:

$$\text{Expected-d}_i = \text{Observed-s}_i \times B_{root-d,i}/B_{root-s,i} \quad (5)$$

(Observed-s_i, observed value for species i in the single-seedling treatment; $B_{root\text{-}d,\ i}$ and $B_{root\text{-}s,\ i}$, root dry biomass of species i in

double- and single-seedling treatment (g pot^{-1}), respectively). If observed values in the double-seedling treatments are lower than expected values, the intraspecific competition effect is negative, and if larger, the effect is positive. By adjusting expected values for root biomass, we reduced possible biases of root bounding (albeit minor; see Fig. S1) on the intraspecific competition effect. Although root biomass has often been used to adjust for root bounding in such competition studies (e.g., Dijkstra *et al.*, 2010; Pausch *et al.*, 2013), we also determined the intraspecific competition effect after adjusting for root length.

We further assessed the magnitude of the potential competition between seedlings using a competition intensity index (CII) (Fort *et al.*, 2014); for a given species i:

$$CII_i = (B_{shoot-s,i} - B_{shoot-d,i})/x \quad (6)$$

($B_{shoot-s}$, shoot dry biomass in single-seedling pots (g per plant); $B_{shoot-d}$, mean shoot dry biomass in double-seedling pots (g per plant); x, maximum of these two values (g per plant)). If CII is larger than zero, it indicates competition.

Finally, we calculated growth rate as the difference in plant biomass between planting and harvest divided by time, and calculated the C : N ratio of SOM mineralization in all treatments as the $C_{soil-derived}$ divided by GNM (Murphy *et al.*, 2015).

Statistical analysis

We used one-way ANOVA with a *post hoc* unequal N Tukey–Kramer honest significant difference (HSD) test to assess effects of species presence and identity (unplanted, planted single seedling and planted double seedlings for each of the three species) on C_{total}, $\delta^{13}C_{total}$, $C_{soil-derived}$, GNM, ratios of SOM mineralization ($C_{soil-derived}$/GNM), MBC, EEAs, net N mineralization and soil mineral N. We used two-way ANOVAs to examine the effects of species identity (larch, ash and Chinese fir), planting density (one and two seedlings), and their interactions on shoot and root biomass, N_{acq}, shoot : root ratio, growth rate, $C_{root-derived}$, primed C, primed N and excess net N mineralization among planted treatments. We used the unequal N Tukey–Kramer HSD test to compare differences among means. Simple linear regressions were used to assess relationships between variables. To determine the (positive or negative) effect of intraspecific competition on primed C, primed N, $C_{root-derived}$, excess net N mineralization, N_{acq}, MBC, EEAs and soil mineral N, we used a two-tailed t-test to examine whether the difference between observed values and expected values deviated from zero. We also used a one-tailed *t*-test to determine whether the CII was larger than zero. All statistical analyses were performed with SPSS 22 and the significance level was set at $P < 0.05$.

Results

Plant biomass, growth rate and net plant N acquisition

When grown as a single seedling, larch had the lowest shoot and root biomass, whereas Chinese fir and ash had the highest shoot biomass and root biomass, respectively (Table 1). In the double-seedling treatment, the ranking of biomass among species remained. However, shoot biomass per plant in the double-seedling treatment was lower than in the single-seedling treatment for ash and Chinese fir, and root biomass per plant was not significantly different between the two treatments (Table 1). The CII suggested significantly intraspecific competition in ash and Chinese fir, but not in larch (Table 1).

Ash and Chinese fir showed four to six times higher growth rates than larch in both density treatments during the experimental period (Table 1). Although all three species showed a declining trend of growth rate in the double-seedling treatment relative to the single-seedling treatment, only Chinese fir showed a significant reduction ($P < 0.05$, Table 1).

The ranking of root, shoot and plant net N acquisition among the three species was similar to the ranking of biomass (Table 1). Notably, the shoot : root ratio of biomass or net N acquisition showed a declining trend in the double-seedling treatment compared to the single-seedling treatment, particularly for Chinese fir ($P < 0.05$, Table 1). This result indicates that intraspecific competition led to proportionally greater allocation of biomass and N to roots, particularly for Chinese fir.

Total soil CO_2 efflux, $\delta^{13}C$ value and soil mineral N

The total soil CO_2 efflux was significantly higher in planted than in unplanted pots, except for the larch single-seedling treatment (Table 2). The $\delta^{13}C$ of total soil CO_2 efflux from the planted treatments varied from −21.9‰ to −23.3‰, and was significantly more depleted compared to the unplanted control treatment (−16.8‰) (Table 2).

Soil mineral N (NH_4^+ plus NO_3^-) was significantly lower in planted treatments than unplanted soil, except for larch (Table 2). The lowest mineral N content was found in the Chinese fir soil, with only *c.* 3% of the mineral N content (unplanted soil) remaining. There were no significant differences in soil mineral N between single-seedling and double-seedling treatments (Table 2).

Soil-derived CO_2, primed C and root-derived CO_2

The six treatments with plants (three species and two densities) all showed higher soil-derived CO_2 compared to the unplanted control treatment (Table 2). Such positive priming effect on soil C mineralization ranged from 26% to 146%. Moreover, the primed C was higher in the double-seedling treatment compared to the single-seedling treatment and differed among the three species (Fig. 1a). Similarly, root-derived CO_2 was higher in the double-seedling treatment than in the single-seedling treatment and differed among the three species (Fig. 1b).

Within each species, primed C was positively related to root-derived CO_2, root biomass, shoot biomass and total biomass, except for root biomass of Chinese fir (Fig. 2). However, the relationships were species-specific, particularly for root and total biomass (Fig. 2). A multiple regression between primed C and biomass variables confirmed the strong relationships (Table S4).

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Table 2 Total soil respiration (C_{total}) and its $\delta^{13}C$ value, soil-derived CO_2 ($C_{soil\text{-}derived}$), gross nitrogen (N) mineralization (GNM), C : N ratio of soil organic matter (SOM) mineralization ($C_{soil\text{-}derived}$: GNM), net N mineralization (NNM), soil mineral N (N_{min}) and microbial biomass C (MBC) at the end of the experiment

Treatment	C_{total} (mg C kg^{-1} soil d^{-1})	$\delta^{13}C_{total}$ (‰)	$C_{soil\text{-}derived}$ (mg C kg^{-1} soil d^{-1})	GNM (mg N kg^{-1} soil d^{-1})	$C_{soil\text{-}derived}$: GNM	NNM (mg N kg^{-1} soil d^{-1})	N_{min} (mg N kg^{-1} soil)	MBC (mg C kg^{-1} soil)
Unplanted control	4.1 ± 0.1^{a}	-16.8 ± 0.4^{c}	4.12 ± 0.1^{a}	0.35 ± 0.0^{a}	11.80 ± 0.2^{c}	$0.19 \pm 0.0^{b,c}$	82.4 ± 1.0^{c}	$198.8 \pm 3.5^{b,c}$
Single-seedling								
Larch	$9.7 \pm 1.3^{a,b,c}$	-21.9 ± 0.5^{b}	5.19 ± 0.3^{a}	$0.84 \pm 0.2^{a,b}$	$6.94 \pm 1.6^{a,b}$	$0.21 \pm 0.0^{b,c}$	72.2 ± 2.8^{c}	$182.1 \pm 6.4^{a,b}$
Ash	13.8 ± 0.6^{b}	$-22.3 \pm 0.2^{a,b}$	$6.53 \pm 0.3^{a,b}$	$0.83 \pm 0.1^{a,b}$	8.08 ± 0.8^{b}	0.25 ± 0.0^{c}	$35.1 \pm 4.8^{a,b}$	172.3 ± 6.4^{a}
Chinese fir	$20.0 \pm 0.6^{b,c}$	$-22.6 \pm 0.2^{a,b}$	9.67 ± 0.5^{c}	$1.37 \pm 0.1^{b,c}$	7.27 ± 0.9^{b}	$0.16 \pm 0.0^{a,b,c}$	2.9 ± 0.4^{a}	208.0 ± 2.7^{c}
Double-seedling								
Larch	13.1 ± 0.4^{b}	$-22.6 \pm 0.1^{a,b}$	$6.32 \pm 0.2^{a,b}$	1.84 ± 0.2^{c}	3.55 ± 0.4^{a}	$0.24 \pm 0.0+$	73.3 ± 2.2^{c}	248.2 ± 7.4^{d}
Ash	20.2 ± 0.6^{c}	-23.3 ± 0.1^{a}	7.64 ± 0.2^{b}	$0.93 \pm 0.1^{a,b}$	$8.44 \pm 0.4^{b,c}$	$0.12 \pm 0.0^{a,b}$	26.7 ± 1.4^{b}	$201.2 \pm 4.3^{b,c}$
Chinese fir	23.9 ± 0.6^{c}	-23.3 ± 0.2^{a}	9.96 ± 0.3^{c}	1.84 ± 0.3^{c}	$5.66 \pm 0.9^{a,b}$	0.08 ± 0.0^{a}	2.5 ± 0.4^{a}	265.3 ± 4.2^{d}

NNM is calculated as changes between final and initial soil inorganic N and plant biomass N pools. For each column, significant differences among unplanted control, single-seedling and double-seedling treatments are denoted by different letters (*post hoc* Tukey–Kramer honest significant difference (HSD) test, $P < 0.05$). Values represent mean ± SE; $n = 4$ for all the treatments except for larch single-seedling treatment where $n = 3$.

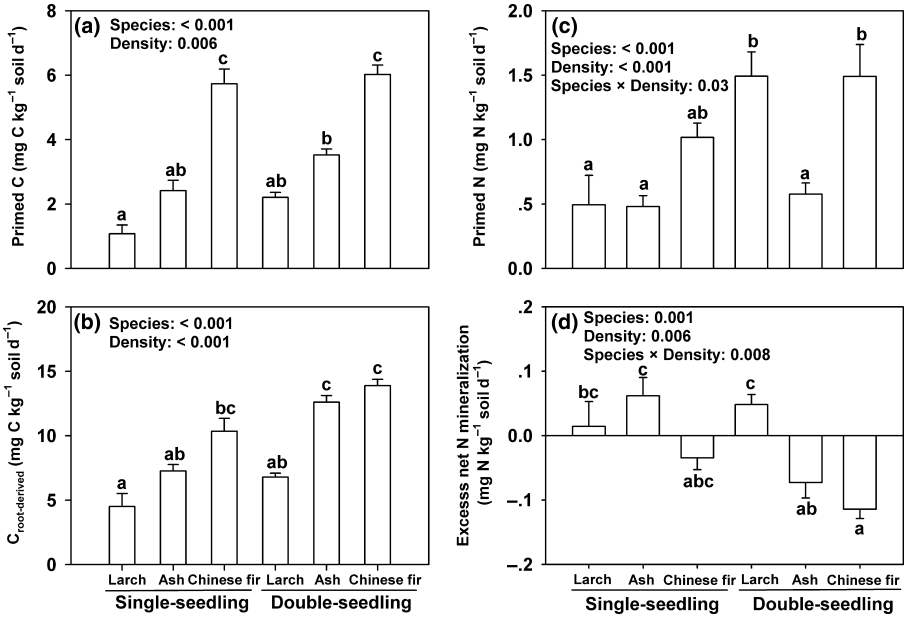

Fig. 1 Primed carbon (C) (the difference in soil-derived CO_2–C between unplanted control and planted treatments (a), root-derived CO_2 flux ($C_{root\text{-}derived}$ (b), primed nitrogen (N) (the difference in gross N mineralization between unplanted control and planted treatments (c) and excess net N mineralization (the difference in net N mineralization between unplanted control and planted treatments (d). Different letters denote significant differences among the six treatments (*post hoc* Tukey–Kramer honest significant difference (HSD) test, $P < 0.05$). Error bars indicate + SE of the mean; $n = 4$ for all treatments except $n = 3$ for larch single-seedling treatment.

GNM, primed N and net N mineralization

Gross N mineralization was also enhanced by living roots of all three species (Table 2). The rhizosphere effect on GNM ranged from 137% to 427%, and the primed N differed among the three species and between the two density treatments (Fig. 1c). Additionally, the C : N ratio of SOM mineralization (i.e., the ratio between soil-derived CO_2 and GNM, Table 2) was reduced in planted treatments (3.6 ~ 8.4) compared to the unplanted control (11.8).

Net N mineralization also was altered by the presence of roots (Table 2). The rhizosphere effect on NNM ranged from −59% to 32%, and the excess NNM differed with species and density (Fig. 1d). Generally, double-seedling treatments showed lower NNM than single-seedling treatments, particularly for ash and Chinese fir.

Correlation among primed C, N and net plant N acquisition

Primed N and net plant N acquisition were positively related to primed C across all treatments (Fig. 3a,c), indicating that the enhanced soil C decomposition by rhizosphere priming was associated with faster soil N mineralization and higher plant N uptake. However, the excess net N mineralization was negatively related to primed C (Fig. 3b).

Soil MBC and extracellular enzyme activities

Compared to the unplanted treatment, MBC was lower in the ash single-seedling treatment (−13%), higher in the larch (32%) and Chinese fir (38%) double-seedling treatments, but not different in other treatments (Table 2). Moreover, MBC was 17%–36% higher in the double-seedling treatment

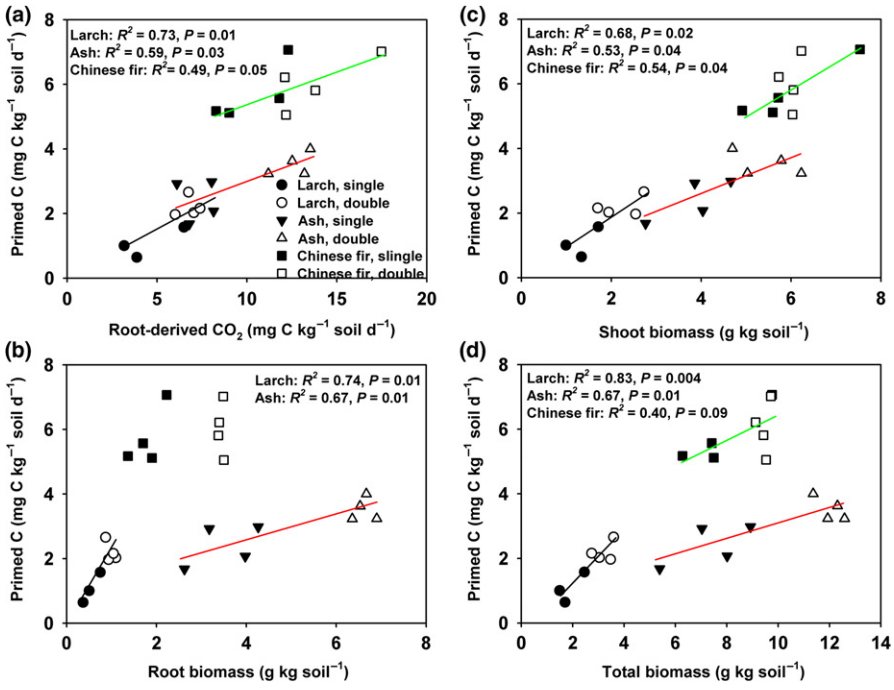

Fig. 2 Linear relationships of primed carbon (C) with root-derived CO_2 flux (a), root biomass (b), shoot biomass (c) and plant total biomass (d). Data for (a), (b), (c) and (d) include single-seedling treatments (closed symbols) and double-seedling treatments (open symbols) of three tree species.

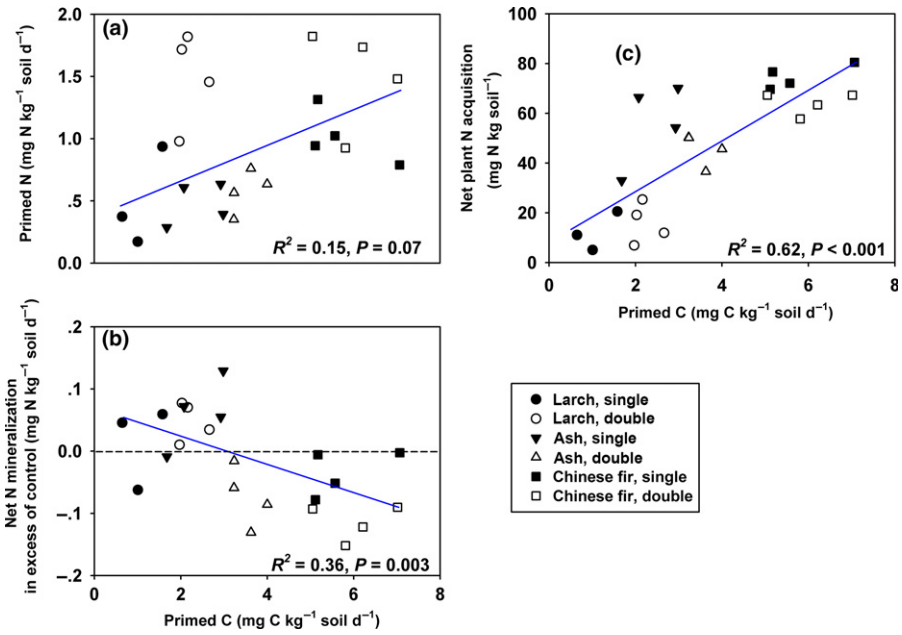

Fig. 3 Linear relationships of (a) primed nitrogen (N) (the difference in gross N mineralization fluxes between unplanted control and planted treatments), (b) excess net N mineralization (the difference in net N mineralization between unplanted control and planted treatments) and (c) net plant N acquisition (plant N content at harvest subtracted by initial plant N content when planted) with primed C (the difference in soil-derived CO_2–C between unplanted control and planted treatments). Data for (a), (b) and (c) include single-seedling treatments (closed symbols) and double-seedling treatments (open symbols) of three tree species.

than the single-seedling treatment across all three species (Table 2).

The activities of two C-acquisition (BG and CB) and two N-acquisition (NAG and LAP) hydrolytic enzymes were mostly similar and sometimes lower (e.g. the Chinese fir double-seedling treatment) in the six planted treatments than in the unplanted control (Fig. S2a,b). However, the total activity of two oxidative enzymes (PO and PER) was higher in three of the six planted treatments compared to the unplanted control (Fig. S2c).

Effects of intraspecific competition

Compared to expected values, the observed primed C decreased for ash and Chinese fir (average decrease of 35%, Fig. 4a), and the observed primed N also decreased for both species (average decrease of 38%, Fig. 4b). This reduction was also found in net plant N acquisition and excess net N mineralization for these two species, with a 71% and 27% decrease compared to expected values, respectively (Fig. 4c,d). These results indicated that primed C, primed N, excess net N

mineralization and net plant N uptake were all significantly reduced by intraspecific competition for these two species, but not for larch (Fig. 4).

Comparisons for other variables showed similar patterns. For example, intraspecific competition tended to reduce root-derived CO_2 (Fig. 5a), microbial biomass C (Fig. 5b), soil mineral N (Fig. 5c) and enzyme activities (Fig. 5d–f), although the effect was minor or even positive for some variables. Furthermore, when we calculated the competition effect after adjusting for root length, the results (Figs S3, S4) were similar to, and mostly more significant than (for larch particularly) the results based on root biomass (Figs 4, 5).

Discussion

Three tree species (larch, ash and Chinese fir) were planted in C_4 soils with one and two seedlings per pot to examine the effects of tree species and intraspecific competition on rhizosphere priming effects (RPEs). We obtained three key findings. First, RPEs on carbon (C) and nitrogen (N) dynamics were mostly positive and differed among tree species. Second, intraspecific competition significantly reduced RPEs, particularly for ash and Chinese fir. Third, microbial mining for N seemed to be the underlying mechanism causing the differences in RPEs across species, whereas competition for N between plants and microbes could largely explain the effects caused by intraspecific competition.

RPE on soil C decomposition

Numerous studies have reported RPEs on soil organic content (SOC) decomposition with crop and grass species as summarized by Cheng *et al.* (2014), but the RPE of tree species on SOC mineralization remains less studied. Using a ^{13}C natural tracer, we showed that RPEs of the three tree species ranged from + 26% to + 146% compared to unplanted soil, with largest RPEs for Chinese fir and lowest for larch (Fig. 1a). The magnitude of these RPEs was comparable to previous results with different tree species and soil types (Bader & Cheng, 2007; Dijkstra & Cheng, 2007; Bengtson *et al.*, 2012).

We also demonstrated significant positive correlations between the 'primed C' and root-derived CO_2, root, shoot and total biomass, respectively, within each species (Fig. 2), which is largely in accordance with previous studies (Dijkstra & Cheng, 2007; Bengtson *et al.*, 2012; Zhu *et al.*, 2014; Wang *et al.*, 2016). For example, Chinese fir had the greatest growth rate and shoot biomass (Table 1), possibly supporting a high rate of root exudation (as indicated by root-derived CO_2, assuming that root-derived CO_2 is proportional to root exudation, Fig. 1b) and subsequently causing the largest primed C (Fig. 1a) (Zhu & Cheng, 2012; Drake *et al.*, 2013). By contrast, larch showed the lowest primed C (Fig. 1a), likely related to its lowest growth rate and shoot biomass (Table 1).

Notably, the relationship between primed C and these biomass variables differed among species, particularly for root and total biomass (Fig. 2), suggesting that the dominant factor controlling RPE may be species-specific. The three tree species differed remarkably in nonbiomass variables (Tables S1, S2), such as mycorrhizal association (arbuscular mycorrhizal vs. ectomycorrhizal fungi) and root traits (e.g. root diameter, specific root length). Such differences may have led to nonuniform relationships between primed C and plant biomass (particularly root and total biomass) across the three species (Fig. 2b,d). Future work should pay attention to the impact of mycorrhizal association (and other plant traits) on RPE by including a large number of tree species with contrasting mycorrhizal associations and plant traits.

RPE on soil N dynamics

Compared to SOC decomposition, RPEs on soil N dynamics have been much less studied and remain poorly understood

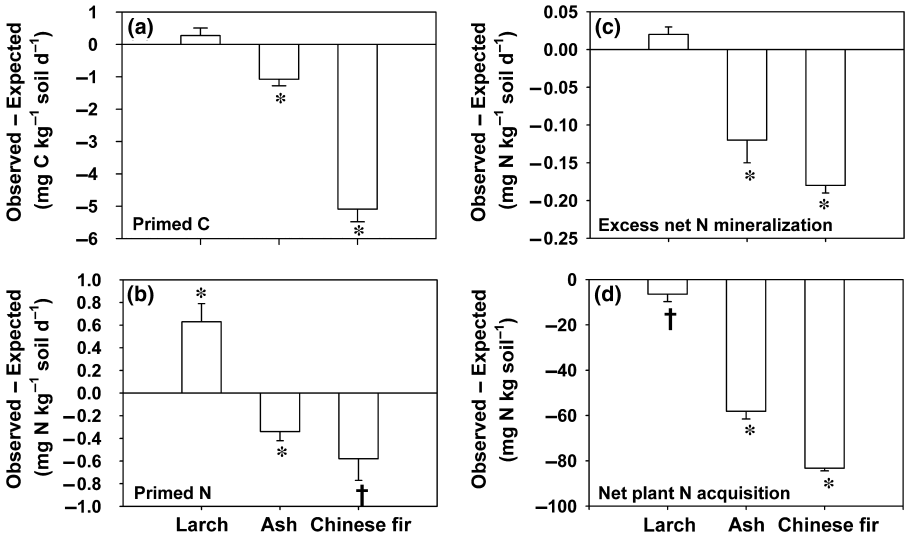

Fig. 4 Observed minus expected values of primed carbon (C, a), primed nitrogen (N, b), excess net N mineralization (c) and net plant N acquisition (d) for larch, ash and Chinese fir double-seedling treatments. Expected values are calculated based on the average values of primed C, primed N, excess net N mineralization and net plant N acquisition of each species in the single-seedling treatment adjusted for root biomass in the double-seedling treatment. Values below zero indicate intraspecific competition. Error bars indicate + SE of the mean. Two-tailed *t*-tests were used to test for significant deviation from zero: †, $0.05 < P < 0.10$; *, $P < 0.05$; $n = 4$ for all treatments.

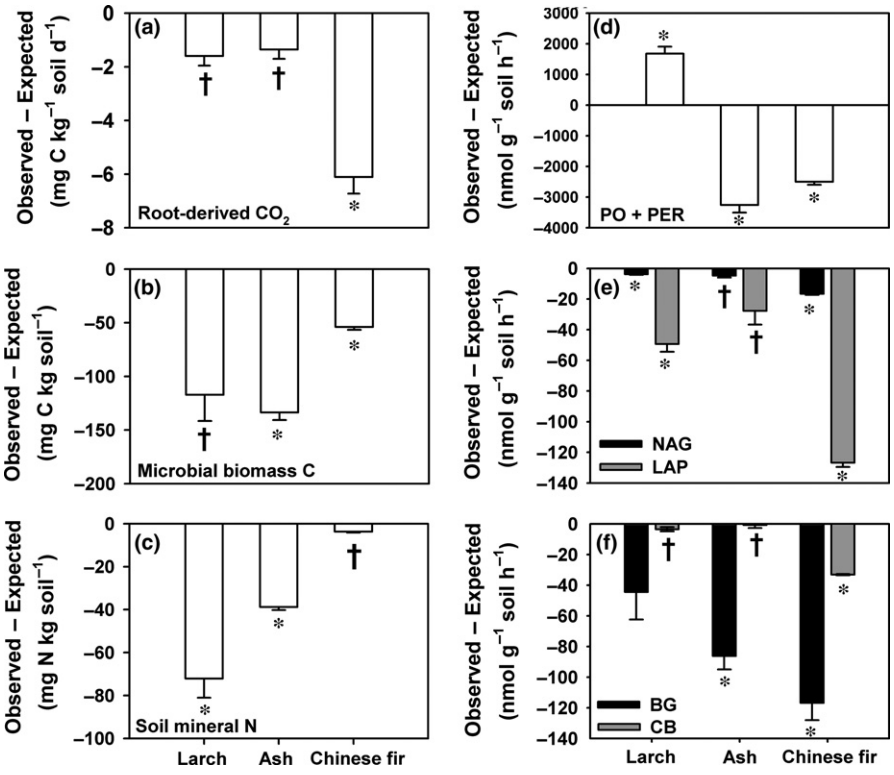

Fig. 5 Observed minus expected values of root-derived CO_2 (a), microbial biomass carbon (C, b), soil mineral nitrogen (N, c), the total activity of phenol oxidase (PO) and peroxidase (PER) (PO + PER, d), N-acetyl-β-glucosaminidase (NAG) and leucin aminopeptidase (LAP) (e) and β-glucosidase (BG) and β-cellobiohydrolase (CB) (f) for larch, ash and Chinese fir double-seedling treatments. Expected values are calculated based on the average values of root-derived CO_2, microbial biomass C, soil mineral N and enzyme activities of each species in the single-seedling treatment adjusted for root biomass in the double-seedling treatment. Values below zero indicate intraspecific competition. Error bars indicate + SE of the mean. Two-tailed *t*-tests were used to test for significant deviation from zero: †, $0.05 < P < 0.10$; *, $P < 0.05$; $n = 4$ for all treatments.

(Frank & Groffman, 2009; Kuzyakov, 2010; Cheng *et al.*, 2014). Here, we found that the primed N ranged from +137% to +427% based on the ^{15}N pool-dilution method (Table 2; Fig. 1c). Similar phenomena also have been observed in other studies with different tree species, soil types and sampling times (Dijkstra *et al.*, 2009; Bengtson *et al.*, 2012). Zhu *et al.* (2014) also observed significant primed N in planted soil in five out of eight treatment combinations with crop species.

The presence of plants did not always result in significantly higher net N mineralization despite positive RPEs both on soil C and gross N mineralization. The excess net N mineralization was always lower than primed N (Fig. 1c,d), suggesting that plants stimulated not only gross N mineralization, but also net soil N immobilization. This discrepancy in primed N and excess net N mineralization varied among the three tree species, where apparent net soil N immobilization was higher for Chinese fir and larch than for ash on average. Similar results were observed by Dijkstra *et al.* (2009) who found that the relationship between primed C and excess net N mineralization was inconsistent among different combinations of tree species and soil types. Moreover, the net plant N acquisition did not follow the excess net N mineralization.

One reason for these discrepancies is that Chinese fir and ash (both associated with arbuscular mycorrhizal (AM) fungi) can promote C decomposition more than N mineralization (Phillips *et al.*, 2013; Cheeke *et al.*, 2017). It is also possible that we underestimated net N mineralization because we ignored potential loss of gaseous N, especially in the AM trees (Chinese fir and ash) that tend to have a more open N cycle than ectomycorrhizal (ECM) trees (larch) (Phillips *et al.*, 2013). We suggest that more combinations of tree species with different mycorrhizal fungi should be considered to investigate the relationship between primed C and net N mineralization in different soil types. Overall, our results (Table 2; Fig. 1) clearly suggest that species-specific effects on primed N, excess net N mineralization and net plant N acquisition should be comprehensively investigated to better understand the apparently decoupled C and N dynamics in the presence of plants (Dijkstra *et al.*, 2009; Zhu *et al.*, 2014).

Mechanism of N mining

The underlying mechanisms of RPE remain largely elusive despite an increasing number of studies focusing on this effect (Cheng & Kuzyakov, 2005; Cheng *et al.*, 2014; Finzi *et al.*, 2015). Our results suggest that plant N uptake depletes mineral N content in the rhizosphere and induces a positive RPE (Fig. 6 left part). When soil mineral N becomes scarce due to plant N uptake (as suggested by a negative relationship between plant total biomass and soil mineral N, Fig. S5a), more quantities of exudates are likely released into the rhizosphere soil (as suggested by the significantly negative correlation between root-derived CO_2 and soil mineral N, Fig. S5b), which further promotes soil C decomposition (Fig. S5c). Consequently, mineralization of soil organic matter (SOM) that is relatively rich in N (as suggested by the relatively lower C : N ratio of *c.* 6.6 of the SOM that was mineralized in planted soil; Table 2) also increases. Similar low

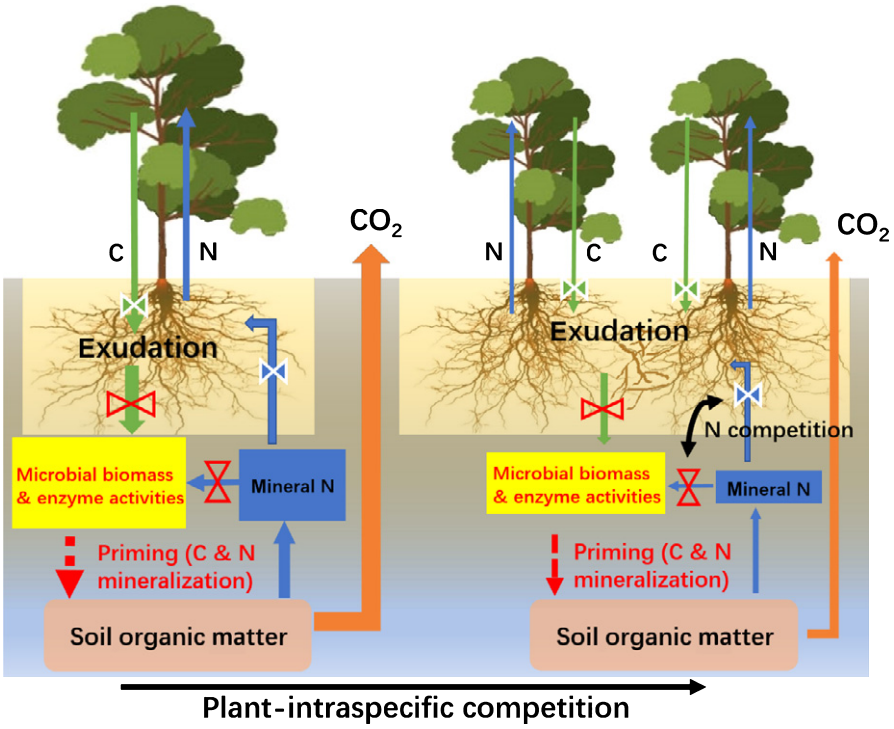

Fig. 6 Conceptual framework depicting mechanisms of rhizosphere priming on soil organic matter (SOM) mineralization. The microbial nitrogen (N) mining hypothesis is shown on the left and the competition hypothesis for reduced rhizosphere priming on carbon (C) and N mineralization on the right. Solid arrows, C and N fluxes from one pool to another; dotted arrows, enzymatic activities that mediate the mineralization of SOM. The thickness of the arrows indicates the magnitude of the flux or activity. Rectangles, the pool sizes of microbial biomass and mineral N (ammonium and nitrate). Green arrows, root exudation; blue arrows, mineral N flow; orange arrows, microbial respiration. Further explanations are shown in the main text.

C : N ratios of SOM mineralization were observed by Murphy *et al.* (2015). As such, tree seedlings in our study caused a positive RPE on SOC decomposition that was tightly coupled with primed N (Fig. 5a) and net plant N acquisition (Fig. 5c).

Therefore, our data support the microbial N mining hypothesis where plants caused an RPE to mine for N from N-rich SOM by stimulating the growth and activity of microbes (Craine *et al.*, 2007). This idea also is supported by several other studies (Dijkstra *et al.*, 2009; Phillips *et al.*, 2011; Bengtson *et al.*, 2012; Zhu *et al.*, 2014; Murphy *et al.*, 2015). Furthermore, compared to the unplanted soil, planting increased the activities of two oxidases responsible for decomposing recalcitrant but N-rich SOM pools (as suggested by the positive correlation between oxidase activities and Gross N mineralization rate (GNM); Fig. S5d) (Zhu *et al.*, 2014; Meier *et al.*, 2015, 2017). These results indicate that, when soil mineral N is depleted, higher microbial investments in enzymes related to the turnover of the slow-cycling SOM pools may trigger the positive RPE. Here we propose that the relationship between C and N mineralization need to be considered together to better understand potential mechanisms of RPEs on the slow-cycling SOM pools (Zhu *et al.*, 2014; Rousk *et al.*, 2016).

Mechanism of N competition

Compared to the single-seedling treatment, the double-seedling treatment did not increase RPE and plant N uptake, even though plant biomass per pot increased significantly by up to 70%. In fact, observed primed C, N, excess net N mineralization and net plant N acquisition with double seedlings of ash and Chinese fir were lower than expected after root biomass (Fig. 4) and length (Fig. S3) calibration, whereas the positive competition intensity index (CII) suggests that intraspecific competition was intense for these two species (Table 1). We suggest that intensified nutrient competition with double seedlings was largely responsible for these lower than expected observations (nutrient competition hypothesis, Fig. 6 right part).

When a seedling occupies the same soil volume as its neighbor, the nutrient demand increases but the nutrient pool stored in SOM does not change. With continued seedling growth and corresponding nutrient uptake, the nutrient pool, and especially the N pool, becomes more and more depleted. With increased intraspecific competition for N, plants may allocate more C to root growth and change root morphological traits (Beyer *et al.*, 2013; Hajek *et al.*, 2014). This could lead to increased competition between plants and microbes, which could result in microbial N limitation (more N taken up by plant roots) (Harrison *et al.*, 2008). Our observations support the competition hypothesis where we observed lower soil mineral N content and lower microbial biomass C and enzyme activities in the double-seedling treatment with ash and Chinese fir compared to the expected values (Figs 5, S4). Meanwhile, root-derived CO_2 was considerably reduced in the double-seedling treatment (Figs 5a, S4a). Consequently, the reduction of RPE also resulted in less net plant N acquisition (Figs 4d, S3d).

Which mechanism dominates and when?

The competition hypothesis appears to contradict the microbial N mining hypothesis that predicts an increase in RPE with depleted soil mineral N driven by microbes mining N-rich SOM. We suggest that the extent to which the soil mineral N is depleted

may act as a critical factor. In light of microbial elemental stoichiometry (Mooshammer *et al.*, 2014), there is a threshold elemental ratio (TER) for microbial growth and activity, which indicates the transition from nutrient limitation to energy (C) limitation (Urabe & Watanabe, 1992; Frost *et al.*, 2006). Thus, with increased N competition between plants and microbes, the microbial C : N ratio may shift to a value greater than the TER, causing microbes to suffer from N stress. Under such circumstances, microbial growth and activities are reduced, resulting in lower C and N mineralization (Schimel & Bennett, 2004; Schimel *et al.*, 2007), which in turn reduces plant N availability and uptake, leading to lower photosynthesis and correspondingly lower exudation.

This explanation is supported by our results. We observed a relatively larger root density (total root biomass per pot divided by soil volume) in the double-seedling treatments (larger overlap of the exploitation spheres), particularly for ash and Chinese fir (mainly due to their higher growth rates), with 92% and 89% increases, respectively. The larger root density suggests a greater capacity to forage for N and compete more intensely with microbes, thereby lowering soil mineral N (Table 2), shoot biomass per plant (Table 1) and root-derived CO_2 (Fig. 1b). Although other studies have shown plant interspecific interactions on the rhizosphere priming effect (Dijkstra *et al.*, 2010; Pausch *et al.*, 2013), we suggest that intraspecific interactions affecting plant–microbe competition for soil mineral N and SOM decomposition also are important (Fig. 6).

Limitations and implications

Our study has two limitations. First, although mycorrhizal types and root traits have been shown to control rhizosphere effects (Phillips & Fahey, 2006; Brzostek *et al.*, 2015), we cannot explicitly link mycorrhizal types and root traits with RPE, because we could only include three tree species in this study due to logistical limitations. Future work should pay attention to the impact of mycorrhizal association and plant traits on RPE by including a large number of species with contrasting mycorrhizal associations and plant traits. Second, the results of this study are not directly applicable to realistic forest ecosystems with mature trees, largely because the method requires the use of a non-native soil (the 'C_4 soil') and small tree seedlings grown in buried pots which inevitably involved artificial conditions significantly different from field ecosystems. New methods for reliably quantifying RPEs in realistic forest ecosystems are needed for future studies (Cheng *et al.*, 2014; Finzi *et al.*, 2015). Knowing these limitations, in this study we specifically focused on identifying underlying mechanisms of RPEs and their response to intraspecific competition.

Our results have important implications for SOM decomposition and N dynamics due to plant–soil interactions and their feedback to forest productivity, considering that intraspecific competition is ubiquitous (large overlap of the exploitation spheres of roots) in the natural environment. Moreover, we showed a strong link between plant biomass, soil mineral N content and SOM mineralization (RPE). Therefore, RPE can play a pivotal role in stimulating N cycling and SOM mineralization, and maintaining plant growth in low-fertility soils. Tree species and intraspecific competition effects on SOM mineralization are relevant for developing tree density-management strategies to maximize forest productivity, minimize nutrient loss and improve organic reserves of nutrients and C in forest plantations. Finally, we obtained quantitative relationships between RPE and plant variables (i.e. plant biomass and root-derived CO_2) among different tree species, which have the potential to improve C- and N-cycling models, and to enhance our capability to predict SOM mineralization and global C stock in a changing world.

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Author contributions

L.Y., P.W., B.Z. and W.C. planned and designed the research; L.Y. performed experiments and analyzed data; and L.Y., F.A.D., P.W., B.Z. and W.C. wrote the manuscript.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information tab for this article:

Fig. S1 Images of representative examples of root systems in the single-seedling and the double-seedling treatments at the end of the experiment.

Fig. S2 Potential extracellular enzyme activities for β-glucosidase (BG, a), β-cellobiohydrolase (CB), N-acetyl-b-glucosaminidase (NAG), leucin aminopeptidase (LAP) and sum of phenol oxidase (PO) and peroxidase (PER) (PO + PER) for unplanted soil, larch, ash and Chinese fir planted as single and double seedlings.

Fig. S3 Observed minus expected values of primed C, primed N, excess net N mineralization and net plant N acquisition for larch, ash and Chinese fir double-seedling treatments.

Fig. S4 Observed minus expected values of root-derived CO_2, microbial biomass C, soil mineral N, the total activity of phenol oxidase (PO) and peroxidase (PER) (PO + PER), N-acetyl-b-glucosaminidase (NAG) and leucin aminopeptidase (LAP) and β-glucosidase (BG) and β-cellobiohydrolase (CB) for larch, ash and Chinese fir double-seedling treatments.

Fig. S5 Linear relationships between total biomass and soil mineral N, between soil mineral N and root-derived CO_2 ($C_{root\text{-}derived}$), between primed C and root-derived CO_2 ($C_{root\text{-}derived}$) and between gross N mineralization and sum of phenol oxidase (PO) and peroxidase (PER) (PO + PER).

Table S1 Seedling biomass and N concentration at planting

Table S2 Morphological traits of absorptive roots and transport roots

Table S3 Comparison of previous studies on RPE of tree species

Table S4 Multiple forward stepwise regression relating primed C to plant biomass and root-derived CO_2

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