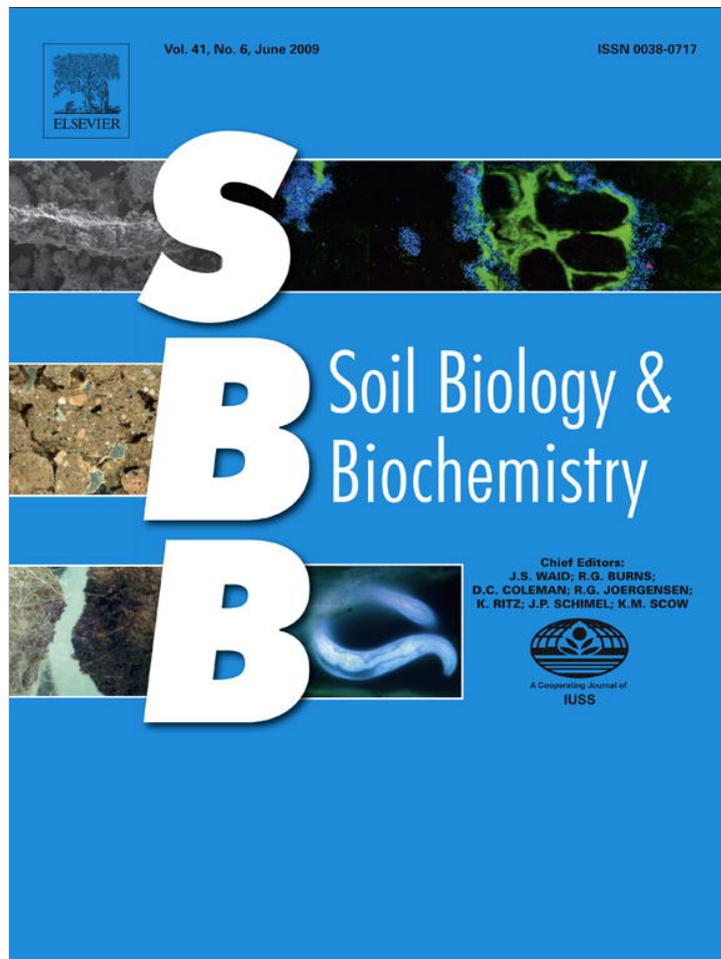


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Does accelerated soil organic matter decomposition in the presence of plants increase plant N availability?

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ABSTRACT

Plant roots can increase microbial activity and soil organic matter (SOM) decomposition via rhizosphere priming effects. It is virtually unknown how differences in the priming effect among plant species and soil type affect N mineralization and plant uptake. In a greenhouse experiment, we tested whether priming effects caused by Fremont cottonwood (*Populus fremontii*) and Ponderosa pine (*Pinus ponderosa*) grown in three different soil types increased plant available N. We measured primed C as the difference in soil-derived CO₂-C fluxes between planted and non-planted treatments. We calculated “excess plant available N” as the difference in plant available N (estimated from changes in soil inorganic N and plant N pools at the start and end of the experiment) between planted and non-planted treatments. Gross N mineralization at day 105 was significantly greater in the presence of plants across all treatments, while microbial N measured at the same time was not affected by plant presence. Gross N mineralization was significantly positively correlated to the rate of priming. Species effects on plant available N were not consistent among soil types. Plant available N in one soil type increased in the *P. fremontii* treatment but not in the *P. ponderosa* treatment, whereas in the other two soils, the effects of the two plant species were reversed. There was no relationship between the cumulative amount of primed C and excess plant available N during the first 107 days of the experiment when inorganic N was still abundant in all planted soils. However, during the second half of the experiment (days 108–398) when soil inorganic N in the planted treatments was depleted by plant N uptake, the cumulative amount of primed C was significantly positively correlated to excess plant available N. Primed C explained 78% of the variability in plant available N for five of the six plant–soil combinations. Excess plant available N could not be predicted from cumulative amount of primed C in one species–soil type combination. Possibly, greater microbial N immobilization due to large inputs of rhizodeposits with low N concentration may have reduced plant available N or we may have underestimated plant available N in this treatment because of N loss through root exudation and death. We conclude that soil N availability cannot be determined by soil properties alone, but that is strongly influenced by root–soil interactions.

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1. Introduction

There is increased recognition that inputs of labile C substrates to the soil can significantly stimulate soil organic matter decomposition (Dijkstra and Cheng, 2007; Fontaine et al., 2007). Plant roots are an important source of labile C (root exudates and other rhizodeposits, Hütsch et al., 2002), and several studies have shown that when plants are present, soil organic matter decomposition

can increase up to 380% compared to soil incubations lacking plants (Cheng et al., 2003). This stimulation of SOM decomposition caused by inputs of labile C substrates has been referred to as the priming effect (Jenkinson et al., 1985; Kuzyakov et al., 2000). Although it has been argued that the priming effect is short-lived and the respired carbon is mostly derived from microbial C pools (Dalenberg and Jager, 1981, 1989; Weintraub et al., 2007), recent studies have shown that a priming effect can be long-lasting (Dijkstra and Cheng, 2007) and can affect old C pools deep in the soil (Fontaine et al., 2007). Increased atmospheric CO₂ concentrations can lead to an increased priming effect because of enhanced availability of labile substrates (Cheng, 1999; Hoosbeek et al., 2004; Trueman and Gonzalez-Meler, 2005). There is an increased awareness that the

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rhizosphere priming effect should be considered as an important mechanism affecting the global C cycle (Heimann and Reichstein, 2008).

The rhizosphere priming effect should also alter N cycling because of the linked nature of C and N decomposition. It has been suggested that the microbial decomposer community in the soil utilizes rhizodeposits as a C source and decompose SOM to acquire N (Kuz'yakov et al., 2000; Paterson, 2003). With priming, more N is then transferred from the inactive SOM pool into the active microbial pool, which eventually could become available for plant uptake (Hungate, 1999; Jones et al., 2004). Rhizodeposition can increase the breakdown of N-rich proteins (Weintraub et al., 2007), while an increase in microbial growth in response to rhizodeposition could further result in increased N availability for plants when these microorganisms are being grazed (Clarholm, 1985). Particularly in soils where inorganic N is scarce, N availability for plants could increase due to an increased priming effect (Cardon, 1996). Herman et al. (2006) reported that N mineralization was up to ten times higher in soil adjacent to *Avena barbata* roots than in bulk soil. Fisher and Stone (1969) long ago noticed increased availability of both N and P in the root zone of conifers and hypothesized that “the conifer rhizosphere mineralizes or otherwise extracts some fraction of the soil nitrogen that had been resistant to microbial action under previous vegetation”. It has also been suggested that greater plant N uptake with defoliation and under elevated CO₂ could be a result of a greater priming effect (Hamilton and Frank, 2001; Martín-Olmedo et al., 2002; Finzi et al., 2007; Phillips, 2007).

The magnitude of the rhizosphere priming effect on SOM decomposition differs among plant species, soil types, and conditions of nutrient availability (Cheng et al., 2003; Fontaine et al., 2004; Dijkstra et al., 2006; Rasmussen et al., 2007). Priming of SOM appears to be greater in dicotyledons than in monocotyledons (Cheng and Kuz'yakov, 2005), smaller in andesitic than in granitic or basaltic soils (Rasmussen et al., 2007), and can become smaller with nutrient addition (Liljeroth et al., 1990; Cardon, 1996; Fontaine et al., 2004), but not always (Cheng et al., 2003). It is virtually unknown how differences in the priming effect among plant species and soil type affect N mineralization and plant uptake.

Earlier we reported a priming effect in all combinations of three different soil types and two tree species (Fremont cottonwood, *Populus fremontii*, and Ponderosa pine, *Pinus ponderosa*) in a greenhouse study (Dijkstra and Cheng, 2007). We determined the priming effect by periodically measuring soil respiration in planted and non-planted control pots and separating soil respiration into plant- and soil-derived CO₂-C using a ¹³C continuous labelling technique. Soil-derived CO₂-C increased up to 225% compared to soil incubations without plants and lasted until the end of the experiment (395 days). The cumulative amount of primed C (i.e., the difference in soil-derived CO₂-C between planted and non-planted control treatments) was larger under *P. ponderosa* than under *P. fremontii* in all three soil types. There were also significant differences among the three soil types that could not be related to total soil C, but may have been a result of differences in soil fertility and mineralogy.

Here we report how the three different soil types and two plant species affected plant N acquisition, microbial N pools, gross N mineralization, and plant available N in this greenhouse experiment. We measured gross N mineralization using the ¹⁵N pool-dilution method (Kirkham and Bartholomew, 1954) and calculated plant available N based on the difference between final and initial inorganic N pools in the soil and the total plant biomass N pool. We hypothesized that gross N mineralization and plant available N would be greater in the planted than in the non-planted control treatments, and that microbial N pools would not be affected by plant presence. We further hypothesized that gross and plant

available N in excess of the control treatments would increase with a greater amount of primed C.

2. Materials and methods

2.1. Experiment setup

We grew Fremont cottonwood (*P. fremontii* S. Wats.) and Ponderosa pine (*P. ponderosa* Douglas ex C. Lawson) in a greenhouse at the University of California, Santa Cruz. During plant growth, the maximum air temperature inside the greenhouse was kept below 27 °C with two cooling units (Mitsubishi Electronics, Lawrenceville, GA). Artificial lighting (1100 W lights, P.L. Light Systems, Beamsville, ON) went on whenever the natural light intensity fell below 200 W m⁻² between 7 AM and 6 PM (Argus Control Systems Ltd., White Rock, BC). The CO₂ concentration was kept at 760 ppm by injecting ¹³C depleted CO₂ in order to label the plants with ¹³C (Dijkstra and Cheng, 2007).

We filled 20 PVC pots (diam. 15 cm, height 40 cm) with 5.0 kg soil obtained from a mixed-conifer forest dominated by *P. ponderosa* at UC Berkeley's Blodgett Forest Research Station in the Sierra Nevada foothills, CA ('Blodgett' soil). We filled 20 pots with 6.8 kg soil obtained from an open oak savanna, dominated by invasive annual grasses at the UC Santa Cruz campus grounds ('UCSC grassland' soil). Both *P. fremontii* and *P. ponderosa* grew nearby in the adjacent UCSC Arboretum. We filled another 20 pots with 5.8 kg soil obtained from a *P. ponderosa* grove on a coastal terrace in West Marshall field, Santa Cruz, part of the UCSC campus reserve ('Marshall field' soil). Soil properties of the three soil types are listed in Table 1. Soils were air-dried and sieved (4 mm) before pots were filled. We planted eight pots of each soil type with *P. fremontii* stem cuttings and eight pots with *P. ponderosa* seedlings that, because of their slow growth from seed, were previously grown for 10 months in small containers (diam. 2.5 cm, height 10 cm). The dry weight of the *P. fremontii* stem cuttings was on average less than 1 g containing less than 7 mg N. The average dry root and shoot weight of 5 separate *P. ponderosa* seedlings measured at the time of planting were 2.1 ± 0.2 and 3.1 ± 0.1 g respectively, with N contents of 25 ± 1 and 40 ± 1 mg respectively (analyzed in the same way as other plant samples, see below). The remaining four pots of each soil type were used as controls. Pots were watered daily to 70% field capacity (no leaching occurred during watering) and were aerated every 6 h for 15 min with an aquarium pump (Apollo AM-3, Apollo Enterprises, Ventura, CA) attached at the bottom of each pot. In this way N loss through leaching/denitrification was insignificant (Cheng, 2008), although some N loss may have occurred when we added ¹⁵N to the pots after 105 days of planting (see below). We harvested four pots of each species of each soil type after 107 days of planting and the other half after 398 days of planting.

2.2. Sampling and analyses

We measured soil respiration on days 24, 60, 100, 156, 206, 296, and 395 after planting. Because plants were labelled with ¹³C, we

Table 1
Soil properties of the three soil types.

	Blodgett	UCSC grassland	Marshall field
Soil class	Ultic Haploxeralf	Pachic Argixeroll	Xeric Argialboll
Texture	Loam	Sandy loam	Loam
pH	5.0	5.0	4.4
Total C (g kg ⁻¹)	52	14	23
Total N (g kg ⁻¹)	2.2	1.2	2.0
C:N	23.7	11.8	11.6

were able to separate plant-derived (root respiration and microbial respiration of rhizodeposits) from soil-derived CO₂-C by measuring the ¹³C signature in soil respiration. A detailed description is given by Dijkstra and Cheng (2007). We calculated 'primed C' as the difference in soil-derived CO₂-C between planted and non-planted control treatments. We calculated the 'cumulative primed C' and the 'cumulative plant-derived CO₂-C' by multiplying the average daily rate of primed C and plant-derived CO₂-C respectively between two measuring dates by the time interval between two measuring dates, and by adding the preceding primed C/plant-derived CO₂-C.

We measured gross N mineralization and nitrification on day 105 using the pool-dilution method (Kirkham and Bartholomew, 1954). To study gross N mineralization we added 10.6 mg of 99.4 atom% ¹⁵N as (NH₄)₂SO₄ and 10.4 mg of unlabelled N as KNO₃ to half of the pots. In the other half of the pots we studied gross nitrification by adding 10.4 mg of 98.0 atom% ¹⁵N as KNO₃ and 10.6 mg of unlabelled N as (NH₄)₂SO₄. Even distribution of the N inside the pots was established by adding the N as a solution to 110% field capacity. Excess solution was pumped from the bottom of the pot into a flask. When the flask was full, the flask was emptied into the top of the pot. This procedure was repeated twice for each pot to ensure adequate dispersion of the ¹⁵N label. Excess solution was drawn into the flask a third time to ensure that the soil was not above field capacity, and the contents of the flask were removed. Soil samples to 20 cm soil depth were taken from each pot directly after labelling and again after 48 h (one 2.5 cm diameter soil core per pot each time to minimize root damage in the pot). After removing roots and recognizable plant material the soil samples were homogenized. A 50 mL solution of 2 M KCl was added to a 10 g subsample, shaken for 1 h, and filtered through pre-leached filter disks (Whatman #1). The KCl extracts were analyzed for NH₄⁺ and NO₃⁻ on a flow injection analyzer (Lachat QuikChem 8000, Milwaukee, WI). For the pots labelled with ¹⁵N as (NH₄)₂SO₄, the NH₄⁺ in the KCl extracts was collected using acidified filter-paper disks inside PTFE diffusion traps (Stark and Hart, 1996). For the pots labelled with ¹⁵N as KNO₃, NH₄⁺ was first removed by adding MgO and letting the samples sit for 5 days. Then, Devarda's Alloy was added to convert the NO₃⁻ into NH₄⁺, which was collected as above. The filter disks were analyzed for total N and δ¹⁵N on a Hydra 20–20 continuous flow isotope mass spectrometer (PDZ Europa, Cheshire, UK). Gross N mineralization and nitrification were calculated using the equations in Kirkham and Bartholomew (1954). For the planted treatments we calculated "excess gross N mineralization" by subtracting the average gross N mineralization in the control treatment of each soil type from the gross N mineralization in the planted treatment with that soil type.

We measured microbial N in the soils collected directly after the ¹⁵N labelling (105 days after planting) using the chloroform fumigation–extraction method (Brookes et al., 1985). A 20 g subsample was immediately added to 50 mL of 0.5 M K₂SO₄, while another 20 g subsample was fumigated with chloroform for 48 h and then added to 50 mL of 0.5 M K₂SO₄. Samples were shaken for 1 h and filtered through pre-leached filter disks (Whatman #1). The organic N in the solution was then oxidized to NO₃⁻ in an autoclave using a persulfate digestion (Cabrerá and Beare, 1993), and the inorganic N was measured on a flow injection analyzer. Microbial biomass N was calculated as the difference between NO₃⁻ concentrations in the fumigated and non-fumigated extracts, divided by the conversion factor 0.54 (Brookes et al., 1985).

After harvesting we separated plant biomass into shoot and root biomass and took a soil sample from each pot. We dried (65 °C), weighed and ground plant biomass from both harvests and dried and ground a subsample of the soil after the final harvest. Ground soil and plant samples were analyzed for total C and N, and δ¹³C on

a Hydra 20–20 continuous flow isotope mass spectrometer (PDZ Europa, Cheshire, UK). After both harvests, we extracted a fresh soil subsample with 2 M KCl as above and analyzed the extracts for NH₄⁺ and NO₃⁻ on a flow injection analyzer (Lachat QuikChem 8000, Milwaukee, WI). We also extracted five pre-treatment soil samples of each soil type with 2 M KCl and analyzed the extracts for NH₄⁺ and NO₃⁻.

We calculated the amount of new root-derived soil organic carbon (root-derived SOC, C_{new}) that was formed during the experiment with:

$$C_{\text{new}} = C_{\text{end}}(\delta^{13}\text{C}_{\text{start}} - \delta^{13}\text{C}_{\text{end}}) / (\delta^{13}\text{C}_{\text{start}} - \delta^{13}\text{C}_{\text{root}}) \quad (1)$$

where C_{end} is the total amount of SOC at the end of the experiment, and δ¹³C_{start}, δ¹³C_{end}, and δ¹³C_{root} are the δ¹³C values of SOC at the start and end of the experiment and of root biomass, respectively.

We calculated net plant N acquisition (N_{acq}, mg N pot⁻¹) by subtracting total N content of the cuttings and seedlings at the time of planting from the total N content of the plant at harvest. We calculated plant available N (N_{pl}, mg N kg⁻¹ soil d⁻¹) separately for the first period (days 0–107) and second period (days 107–398) as follows:

$$N_{\text{pl}} = \left[N_{\text{acq}} / \text{Soil}_{\text{wt}} + \left(\text{InorgNsoil}_{\text{final}} - \text{InorgNsoil}_{\text{initial}} \right) \right] / \Delta\text{time} \quad (2)$$

where Soil_{wt} is the soil dry weight of the pot (kg pot⁻¹), InorgNsoil_{final} is the sum of extractable NH₄⁺ and NO₃⁻ at the time of harvesting (at days 107 and 398 for the first and second period respectively, mg N kg⁻¹ soil), InorgNsoil_{initial} is the sum of extractable NH₄⁺ and NO₃⁻ at the start of each period (from pre-treatment soil samples and at day 107 for the first and second period respectively, mg kg⁻¹ soil), and Δtime is the time interval of each period (107 and 291 days for the first and second period respectively). Some of the N in N_{acq} may have been taken up in organic form (*P. ponderosa* in particular because of its association with ectomycorrhizae). Thus, plant available N is the sum of net N mineralization and organic N uptake. As mentioned above, N loss through leaching/denitrification was insignificant. Because we added a total of 21 mg N to each of the pots on day 105 (just before the first harvest) we included 21 mg N to InorgNsoil_{initial} for calculating N_{min} during the first period. When we discarded excess solution pumped from the bottom of the pots after the N was added we may also have removed some of the N that was in the pots. Therefore, the plant available N calculated for the first period may have been slightly underestimated (we did not measure the inorganic N concentration in the excess solution). For the planted treatments we calculated "excess plant available N" by subtracting the average total amount of plant available N (mg N kg⁻¹ soil) in the control treatment of each soil type from the total amount of plant available N in the planted treatment with that soil type.

We used analysis of variance (ANOVA) to test for main effects of soil type (Blodgett, UCSC grassland, Marshall field) and plant treatment (control, *P. fremontii*, and *P. ponderosa*) and for soil type × plant treatment interactions on plant biomass, new root-derived SOC, net plant N acquisition, inorganic N pools in the soil, gross N mineralization, nitrification, microbial N, and plant available N. We used post-hoc tests (Tukey's test) for each soil type to test for differences in gross N mineralization, microbial N, and plant available N among the two tree species and the control pots. We used linear and non-linear regressions to relate excess gross N mineralization to primed C at day 100 and excess plant available N to cumulative primed C and cumulative plant-derived CO₂-C. We used the software package JMP 4.0.4 for all our statistical analyses.

3. Results

Across soil types, *P. ponderosa* had significantly larger root biomass than *P. fremontii* at both harvests, but significantly larger shoot biomass only at the first harvest (Table 2). Because we started with 10-month-old *P. ponderosa* seedlings that were bigger in size than the *P. fremontii* cuttings, this head start may have contributed to greater root biomass for *P. ponderosa* at both harvests and greater shoot biomass at the first harvest. However, we expected *P. fremontii* in general to be a faster growing species than *P. ponderosa*, which was corroborated by similar or slightly higher shoot biomass for *P. fremontii* than for *P. ponderosa* by the end of the second harvest. There were significant soil type effects on shoot and root biomass and sometimes significant soil type \times plant species interactions. These effects were not consistent for the two harvests, e.g., shoot biomass was highest in the UCSC grassland soil during the first harvest, but highest in the Blodgett soil during the second harvest. Root biomass showed large increases between the first and second harvest, except for *P. fremontii* in the Blodgett and Marshall field soils. New root-derived SOC at the second harvest was on average significantly higher in soils with *P. ponderosa* than in soils with *P. fremontii*. As with plant biomass, new root-derived SOC may have been higher in soils with *P. ponderosa* than with *P. fremontii* because we started the experiment with 10-month-old seedlings. However, the root biomass of the 10-month-old *P. ponderosa* seedlings we started with was on average only 2.1 g or less than 8% of the root biomass by the end of the experiment. The Blodgett soil with *P. ponderosa* plants had the highest new root-derived SOC of all soil type and plant species combinations.

Across soil types, net plant N acquisition in roots was significantly larger for *P. ponderosa* than for *P. fremontii* after both harvests (Table 3). On the other hand, net N acquisition in shoots was significantly higher for *P. fremontii* than for *P. ponderosa* across soil types after the first harvest and almost 100% higher in the Blodgett soil after the second harvest. Total net plant N acquisition was on average not significantly different between *P. fremontii* and *P. ponderosa* during the first harvest, and was on average significantly higher for *P. ponderosa* than for *P. fremontii* during the second harvest. Net plant N acquisition showed significant soil type \times plant species interactions. Notably, during the second harvest *P. ponderosa* had a higher total net plant N acquisition in the UCSC grassland and Marshall field soil, but a lower total net plant N acquisition in the Blodgett soil than *P. fremontii*, despite the fact that the priming effect was largest in the Blodgett soil with *P. ponderosa* among all soil type and plant species combinations (Dijkstra and Cheng, 2007).

The UCSC grassland soil had the highest initial (pre-treatment) NO_3^- and the lowest NH_4^+ content (Table 4). The NO_3^- and total inorganic N content in the control pots increased over time because of net N mineralization and nitrification. In the planted pots NH_4^+ and NO_3^- decreased over time, indicating that the plants took up the inorganic N that was mineralized as well as the inorganic N that was already available at the start of the experiment. After the second harvest relatively little NH_4^+ and NO_3^- were left in the planted pots, except for *P. fremontii* in the Marshall field soil, which had the highest NO_3^- concentration. This soil–plant combination also had the lowest new root-derived SOC (Table 2).

Gross N mineralization measured on day 105 was not significantly affected by soil type, but was significantly affected by plant treatment (Fig. 1A). All planted pots showed on average higher gross N mineralization rates than the control pots, although only *P. ponderosa* in the Blodgett and UCSC grassland soil had significantly higher gross N mineralization rates than their controls. Notably, the Blodgett soil with *P. ponderosa* that showed the largest priming effect also had the greatest gross N mineralization rate at day 105. We observed no significant treatment effects on gross nitrification (data not shown).

The microbial N pool measured on day 105 was significantly greater in the Marshall field soil than in the other two soil types (Fig. 1B). In contrast to the gross N mineralization rate, microbial N was not significantly affected by the plant treatment.

Plant available N calculated according to equation (2) was similar in the planted and the control pots during the first 107 days (Fig. 2), except for *P. ponderosa* in the Marshall field soil, which showed lower plant available N than the control. During the last 291 days plant available N was significantly higher for *P. fremontii* in the Blodgett soil and significantly higher for *P. ponderosa* in the UCSC grassland and Marshall field soils than in the control pots. In general, plant available N was lower during the last 291 days than during the first 107 days.

When we related the excess gross N mineralization (gross N mineralization in planted pots minus average gross N mineralization in control pots) measured on day 105 to the rate of primed C measured on day 100 we observed a weak but significant positive linear relationship (Fig. 3A). A similar relationship was found between gross N mineralization on day 105 and soil-derived $\text{CO}_2\text{-C}$ measured on day 100 ($P = 0.05$, $R^2 = 0.21$). As can be inferred from Fig. 2, excess plant available N was not always positive. During the first period we observed no significant relationship between excess plant available N and the cumulative primed C ($P = 0.92$, $R^2 = 0.0005$, data not shown). During the second period the relationship was marginally significant ($P = 0.08$, $R^2 = 0.17$ for

Table 2
Average plant biomass (± 1 SE) at the first and second harvest and new root-derived SOC at the second harvest.

Soil type	Plant species	First harvest			Second harvest			New root-derived SOC (g C pot ⁻¹)
		Biomass (g pot ⁻¹)			Biomass (g pot ⁻¹)			
		Roots	Shoots	Total	Roots	Shoots	Total	
Blodgett	<i>P. fremontii</i>	2.9 \pm 0.8	6.6 \pm 1.0	9.5 \pm 1.8	12.8 \pm 1.6	25.1 \pm 0.8	37.8 \pm 2.2	3.4 \pm 0.2
	<i>P. ponderosa</i>	11.1 \pm 0.5	12.6 \pm 0.7	23.7 \pm 1.2	26.4 \pm 3.4	19.6 \pm 1.4	45.9 \pm 4.8	9.2 \pm 1.4
UCSC grassland	<i>P. fremontii</i>	2.5 \pm 0.4	13.0 \pm 3.1	15.5 \pm 3.4	25.1 \pm 0.8	23.8 \pm 0.8	48.9 \pm 1.1	4.9 \pm 1.1
	<i>P. ponderosa</i>	9.7 \pm 1.3	12.9 \pm 1.8	22.5 \pm 3.1	28.5 \pm 4.9	20.3 \pm 1.1	48.8 \pm 6.0	5.8 \pm 0.6
Marshall field	<i>P. fremontii</i>	2.2 \pm 0.3	7.1 \pm 0.7	9.3 \pm 1.0	8.7 \pm 1.3	17.1 \pm 2.6	25.8 \pm 2.6	2.7 \pm 0.3
	<i>P. ponderosa</i>	7.2 \pm 0.6	9.9 \pm 1.3	17.0 \pm 1.5	32.5 \pm 2.7	18.5 \pm 1.7	51.1 \pm 4.3	4.1 \pm 1.2
ANOVA <i>P</i> -values								
Soil type		0.02	0.04	0.05	0.03	0.02	0.04	0.02
Plant species		<0.0001	0.05	<0.0001	<0.0001	0.06	0.002	0.002
Soil type \times plant species		0.10	0.20	0.22	0.004	0.10	0.01	0.03

Table 3
Average net plant N acquisition in plant biomass (± 1 SE) at the first and second harvest (plant N content at harvest minus initial plant N content when planted).

Soil type	Plant species	First harvest			Second harvest		
		Root N (mg pot ⁻¹)	Shoot N (mg pot ⁻¹)	Total N (mg pot ⁻¹)	Root N (mg pot ⁻¹)	Shoot N (mg pot ⁻¹)	Total N (mg pot ⁻¹)
Blodgett	<i>P. fremontii</i>	31 \pm 5	127 \pm 19	158 \pm 23	145 \pm 28	348 \pm 18	493 \pm 45
	<i>P. ponderosa</i>	122 \pm 12	130 \pm 10	252 \pm 20	208 \pm 28	175 \pm 9	382 \pm 36
UCSC grassland	<i>P. fremontii</i>	25 \pm 3	206 \pm 33	230 \pm 37	222 \pm 13	182 \pm 20	403 \pm 16
	<i>P. ponderosa</i>	102 \pm 15	101 \pm 12	203 \pm 20	274 \pm 43	194 \pm 45	468 \pm 87
Marshall field	<i>P. fremontii</i>	22 \pm 2	122 \pm 12	144 \pm 13	145 \pm 20	191 \pm 36	336 \pm 49
	<i>P. ponderosa</i>	61 \pm 12	76 \pm 6	137 \pm 17	419 \pm 44	227 \pm 12	646 \pm 54
ANOVA <i>P</i> -values							
Soil type		0.006	0.02	0.008	0.008	0.02	0.44
Plant species		<0.0001	0.003	0.30	<0.0001	0.05	0.04
Soil type \times Plant species		0.04	0.02	0.04	0.003	0.0007	0.001

cumulative primed C). However, when we excluded the Blodgett soil with *P. ponderosa* that showed among the highest cumulative amounts of primed C but that had negative excess plant available N, then the relationship became highly significant, and the relationship increased exponentially (Fig. 3B).

4. Discussion

During the first 107 days of the experiment the presence of plants resulted in increased SOC decomposition and gross N mineralization, but did not affect plant available N and microbial N pools. These results suggest that the priming effect during the first 107 days increased microbial N turnover, but not N availability for plants. Because roots did not fully occupy the pots before the first harvest, the plants may have competed less for N with microbes during this period. Inorganic N was still relatively high during this period, so that plant N demand may not have been limited by microbial N mineralization during this period. The relatively high soil inorganic N during the first period of the experiment may also have reduced the priming effect (Liljeroth et al., 1990; Cardon, 1996). The amount of primed C during the first period was on average 0.3 g C kg⁻¹ soil or 13% of the amount of primed C during the whole experiment (Dijkstra and Cheng, 2007).

During the second part of the experiment (days 108–398) the presence of plants increased plant available N in only three of the six soil type and plant species combinations. However, these differences in plant available N can in a large part be explained by the differences in the cumulative amount of primed C among the six plant–soil combinations. With the exception of the Blodgett soil

with *P. ponderosa* plants, there was a significant positive relationship between excess plant available N and the cumulative amount of primed C during the second period. The plant–soil combinations of *P. ponderosa* in the UCSC grassland and Marshall field soils largely drove the relationship between excess plant available N and the cumulative amount of primed C, which had also among the largest priming effects. However, the cumulative amount of primed C explained 78% of the variability in plant available N when five of the six plant–soil combinations were included in the regression. Increased net N mineralization has been inferred from a priming effect in other studies (Hamilton and Frank, 2001; Martín-Olmedo et al., 2002). Here we show that the increase in plant available N (including gross and net N mineralization) in the presence of a plant can be directly linked to the magnitude of the priming effect in terms of C mineralization.

The excess plant available N in the Blodgett soil with *P. ponderosa* plants was much lower than predicted from the relationship with cumulative primed C. The Blodgett soil had over three times more labile C and a C:N ratio more than double that of the UCSC grassland and Marshall field soils (Table 1, Dijkstra and Cheng, 2007), which could have kept microbial N immobilization high and net N mineralization low. However, despite a much lower amount of cumulative primed C (Dijkstra and Cheng, 2007, Fig. 3B) and a potential lag in growth because we started with cuttings rather than 10-month-old seedlings, plant available N in the Blodgett soil planted with *P. fremontii* was significantly higher than in the non-planted control treatment. This suggests that the low plant available N in the Blodgett soil with *P. ponderosa* was not solely due to soil characteristics.

Table 4
Average soil inorganic N before planting (pre-treatment) and at the first and second harvest (± 1 SE).

Soil type	Plant treatment	Pre-treatment			First harvest			Second harvest		
		NH ₄ ⁺ (mg pot ⁻¹)	NO ₃ ⁻ (mg pot ⁻¹)	Total (mg pot ⁻¹)	NH ₄ ⁺ (mg pot ⁻¹)	NO ₃ ⁻ (mg pot ⁻¹)	Total (mg pot ⁻¹)	NH ₄ ⁺ (mg pot ⁻¹)	NO ₃ ⁻ (mg pot ⁻¹)	Total (mg pot ⁻¹)
Blodgett	Control	31 \pm 3	40 \pm 10	71 \pm 8	14 \pm 3	254 \pm 19	268 \pm 20	3 \pm 0	431 \pm 50	434 \pm 50
	<i>P. fremontii</i>				18 \pm 3	80 \pm 15	97 \pm 17	5 \pm 1	7 \pm 2	12 \pm 2
	<i>P. ponderosa</i>				15 \pm 2	27 \pm 8	42 \pm 10	2 \pm 0	0 \pm 0	2 \pm 0
UCSC grassland	Control	8 \pm 1	139 \pm 20	147 \pm 20	18 \pm 2	229 \pm 48	247 \pm 48	3 \pm 1	323 \pm 51	326 \pm 51
	<i>P. fremontii</i>				22 \pm 5	46 \pm 14	68 \pm 17	2 \pm 0	20 \pm 6	22 \pm 6
	<i>P. ponderosa</i>				13 \pm 1	27 \pm 7	40 \pm 7	3 \pm 0	3 \pm 1	6 \pm 1
Marshall field	Control	41 \pm 6	70 \pm 3	111 \pm 8	30 \pm 3	232 \pm 2	263 \pm 4	8 \pm 1	454 \pm 48	462 \pm 48
	<i>P. fremontii</i>				28 \pm 4	89 \pm 12	117 \pm 14	5 \pm 1	47 \pm 13	52 \pm 12
	<i>P. ponderosa</i>				22 \pm 3	28 \pm 3	50 \pm 5	5 \pm 0	1 \pm 0	6 \pm 0
ANOVA <i>P</i> -values										
Soil type		0.0001	0.0005	0.005	0.0003	0.27	0.20	<0.0001	0.13	0.11
Plant treatment					0.04	<0.0001	<0.0001	0.11	<0.0001	<0.0001
Soil type \times plant treatment					0.64	0.49	0.70	0.005	0.16	0.16

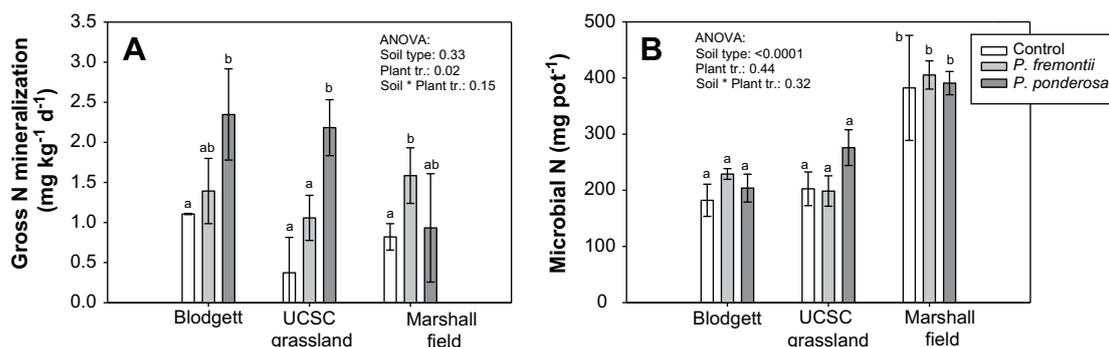


Fig. 1. Gross N mineralization (A) and microbial N (B) averaged by soil type and tree species measured 105 days after transplanting. Different letters for each soil type within each period denote differences among tree species and control treatments (post-hoc Tukey's test, $P < 0.05$).

One potential explanation for the relatively low plant available N in the Blodgett soil with *P. ponderosa* is that decomposition of rhizodeposits may have stimulated microbial N immobilization. Rhizodeposits tend to have a lower N content than older native SOM (Hungate, 1999). The Blodgett soil with *P. ponderosa* had the highest new root-derived SOC at the end of the experiment (two to three times higher than in the other treatments). The presence of new root-derived SOC at the end of the experiment indicates that these rhizodeposits resisted decomposition until the termination of the experiment, although they also may have been incorporated into microbial biomass. Decomposition of N-poor rhizodeposits may have increased microbial N immobilization. Thus, in the Blodgett soil with *P. ponderosa*, increased microbial N immobilization because of increased decomposition of new root-derived SOM may have offset the positive effects of priming on net N mineralization.

Another potential explanation for the low plant available N in the Blodgett soil with *P. ponderosa* may be that we underestimated plant available N in the planted treatments. Nitrogen that was mineralized, taken up by the plant and subsequently lost by the plant through root exudation, ectomycorrhizal turnover, and root death during the experiment was not included in the calculation. The substantial amounts of root-derived SOC in all planted treatments by the end of the experiment (Table 2) indicate that root exudation and death were substantial during the experiment, which most likely also resulted in plant N loss. Others have suggested that plants can lose a substantial amount of N through rhizodeposition (De Graaff et al., 2007; Wichern et al., 2008). As mentioned above, the root-derived SOC was largest for the Blodgett soil with *P. ponderosa* plants, suggesting that rapid root and

mycorrhizal turnover may have led us to underestimate plant available N for this treatment.

The exponential increase in excess plant available N with increased cumulative primed C during the second period indicates that more excess N was mobilized per unit of primed C at greater rates of priming. Also, the priming effect on SOM decomposition increased at the end of the experiment (Dijkstra and Cheng, 2007) at a time when inorganic N content in the soil was low (Table 4). Roots fully occupied the pots by this time and may have competed for N with microbes more effectively during the second period. Thus, the priming effect on SOM decomposition may release more N from soil pools for plant N uptake at a time when the inorganic N content in the soil is low, and when plant N demand is high. This is in accordance with the conceptual model proposed by Cardon (1996) and Hungate (1999) who suggested that when soil nutrients are scarce, microbes use rhizodeposits to breakdown soil organic matter thereby moving nutrients to the active microbial pool, where eventually these nutrients should become available for plants. Others have suggested that the nutrient status of the soil is an important factor controlling the priming effect (Liljeroth et al., 1990; Fontaine et al., 2004) and rates of N mineralization and immobilization (Chapin et al., 2002; Phillips and Fahey, 2008).

Our results suggest that the increase in SOM decomposition caused by rhizosphere priming effects can sometimes increase N mineralization and plant N uptake. However, the relationship between priming and plant available N was not straightforward, and may also depend on the balance between rhizodeposits used for SOM decomposition and rhizodeposits used for microbial growth. On the one hand, rhizodeposits could stimulate N-rich SOM decomposition and enhance net N mineralization while on the

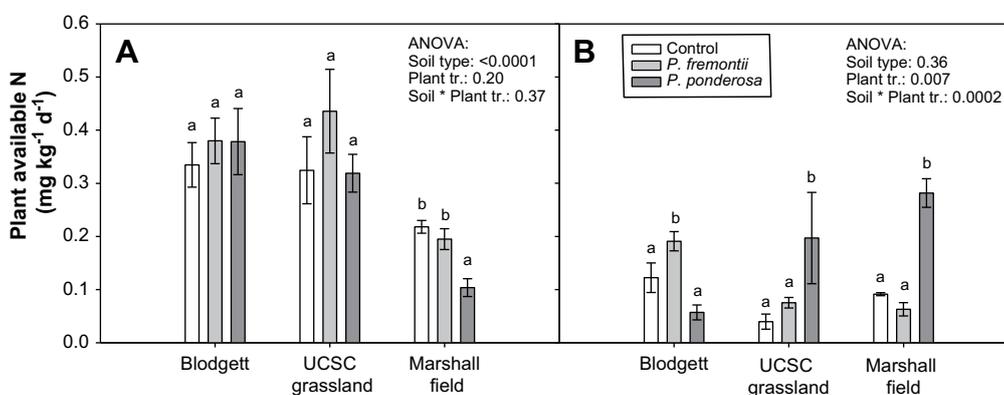


Fig. 2. Plant available N averaged by soil type and tree species during the first (A) and second period (B). Different letters for each soil type within each period denote differences among tree species and control treatments (post-hoc Tukey's test, $P < 0.05$).

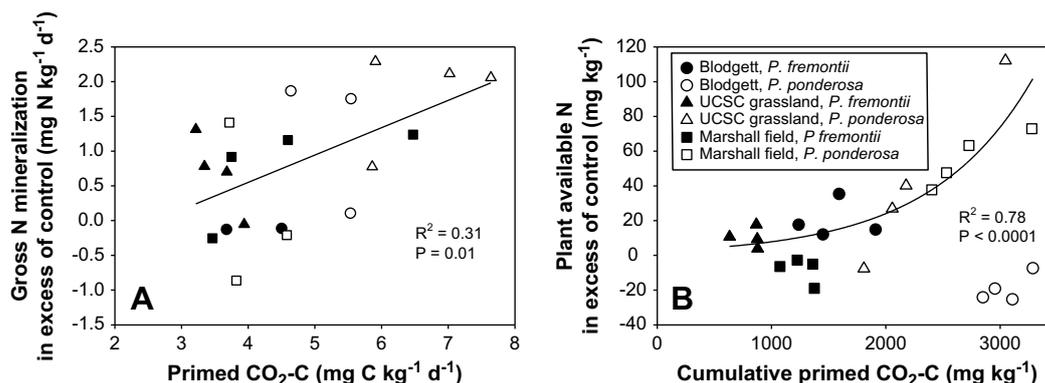


Fig. 3. Excess gross N mineralization as a function of primed C 105 days after transplanting (A) and excess plant available N as a function of cumulative primed C during the second period (B) for each soil type and tree species. Best-fit line in (B) excludes the *P. ponderosa* – Blodgett soil pots (see Discussion).

other hand decomposition of N-poor rhizodeposits could enhance microbial N immobilization (similar to the concept that priming effects on SOM decomposition depend on the stimulation of slow growing *K*-strategist microorganisms vs. fast growing *r*-strategist microorganisms, Fontaine et al., 2003). Because we did our experiment under elevated CO₂ conditions (roughly twice ambient CO₂ concentration), rhizosphere priming effects may have been larger than under ambient CO₂ concentration (Cheng, 1999), and the effects of priming on N cycling may also have been different. Nevertheless, our results suggest that ‘soil N availability’ and ‘soil fertility’ are relative terms, and that N availability cannot be determined by soil properties alone, but is strongly influenced by root–soil interactions.

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