



Responses of microbial activity to carbon, nitrogen, and phosphorus additions in forest mineral soils differing in organic carbon content

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Abstract

Labile C input to the soil can cause the priming effect (PE) that in turn changes the soil organic C (SOC) content. However, little information is available to predict the magnitude of the PE in different soils, especially under concurrent changes in nutrient inputs. We took advantage of a natural gradient in labile C input in the surroundings of wood ant nests in a temperate coniferous forest which arises through the long-term effects of wood ant foraging on the inputs of honeydew to soil. We collected soils from the surface mineral horizon (high-SOC content) (A horizon) and the subsoil mineral horizon (low-SOC content) (B horizon) at 4 m (low labile C input and higher SOC content) and 70 m (high labile C input and lower SOC content) from four nests. In a 6-month laboratory microcosm experiment, we monitored microbial activity and PE as affected by no nutrient addition (control) or fortnightly additions of labile C alone or in combination with N and/or P (C, CN, CP, CNP). Microbial activity and PE after C addition increased more at 70 m than at 4 m in the B horizon, that is, were higher with a lower SOC content. However, microbial activity and PE in the B horizon were not affected by additions of N and/or P with C. In the A horizon, microbial activity and PE were lower after combined CN addition but increased by combined CP addition relative to C addition alone. In conclusion, labile C inputs had a larger effect on decomposition and PE in low-SOC than high-SOC soils, whereas N and P inputs had greater effects in high-SOC soils than in low-SOC soils. This suggests that low-SOC soils such as those subjected to a high long-term labile C input or those from the subsoil mineral horizon might be more susceptible to increase microbial activity in relation to changes in labile C inputs but less susceptible in relation to changes in N and P inputs relative to high-SOC soils.

Keywords Labile C input gradient · *Picea abies* · Nutrients · Deep soil · Soil respiration · Sugarcane sucrose

Introduction

Temperate forest soils represent significant pools of soil organic C (SOC) (Lal 2008; Tyrrell et al. 2012). Because soil microbial communities are generally C-limited (Demoling et al. 2007), fresh labile C input in the form of litter leachates or root exudates can enhance SOC decomposition leading to SOC losses through increased microbial respiration, i.e., through the priming effect (PE) (Kuzyakov et al. 2000). The

extent to which labile C stimulates PE differs among soils depending on the availability of SOC and nutrients for microbial populations (Paterson and Sim 2013). Such differences, however, have not been investigated in temperate coniferous forest soils, especially with a view of changes in SOC content connected with changes in labile C input. Moreover, previous studies typically used a single addition of a high amount of labile C rather than repeated additions of low amounts of labile C; the latter approach results in a much lower PE and is more ecologically representative (Wang et al. 2019). In the current study, we determined how the PE is affected by the fortnightly input of low levels of C and nutrients to temperate coniferous forest soils that differ in SOC content.

The SOC content of mineral soils in temperate coniferous forests is almost four times higher in the surface mineral horizon (0–10 cm) than in the mineral subsoil (50–60 cm) (Jobbágy and Jackson 2000; Jílková et al. 2020a, b). Labile C input is also much greater in surface than in subsoil horizons, such that the deeper layers are energy poor (Fontaine

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et al. 2007). Both the SOC content, which serves as the C source for PE (Kuzuyakov et al. 2000), and the labile C input, which is the major form of C used by microbes (Kemmitt et al. 2008), affect the strength of the PE. As a result, deeper soil layers are subject to a stronger PE than surface soils when labile C is added even though the deeper soils contain less SOC (Wang et al. 2015).

Soils receiving continuous high labile C inputs usually have a reduced SOC content (Jilková et al. 2020a, b). Such a situation may arise in temperate forest ecosystems due to changes in labile C input in throughfall as recently documented by Jilková et al. (2020a). Soils receiving continuous labile C inputs may have shifted the microbial community so that it contains relatively high levels of microbes that are better able to utilize labile C inputs than to decompose SOC (Fontaine et al. 2003; Paterson et al. 2009; Guenet et al. 2010). We therefore expect that the response of PE to labile C additions will be weaker in soils that typically receive high rather than low inputs of labile C.

Nitrogen (N) and phosphorus (P) play a crucial role in regulating the PE (Fontaine et al. 2011; Dijkstra et al. 2013; Chen et al. 2014). Despite high nutrient depositions, most temperate coniferous forest soils are not saturated by N (Du et al. 2019), and are even limited by P (Batjes 1997). The addition of these nutrients to soils might thus increase microbial activity and SOC decomposition. To explain these increases, researchers have proposed several theories. Hessen et al. (2004) suggest that microbial activity is constrained by the scarcest nutrient and that addition of that nutrient will increase SOC decomposition. Because the subsoil mineral horizon is largely C-limited, we might expect that SOC decomposition will be affected more by addition of C than by addition of other nutrients. Similarly, addition of low levels of N to soils might help microbes alleviate N limitation and thus allows microbes to use the added C to produce enzymes to decompose SOM (Drake et al. 2013). Such a situation may arise when the C:N ratio is high, which is usual in the A horizon (Rumpel and Kögel-Knabner 2011). However, when higher levels of N are provided, a lower PE would occur because the microbial community would utilize the added nutrients rather than obtaining those nutrients by decomposing the SOM (Chen et al. 2014). On the other hand, the addition of P to soil should not be directly related to the SOM mining because P is not necessarily associated with SOM (Craine et al. 2007; Milcu et al. 2011). Instead, P might serve as a nutrient for microbial metabolism, and thus, the addition of P could lead to an increase in microbial activity and potentially to an increase in the PE. However, most previous studies examined SOC decomposition after additions of separate nutrients (e.g., Wild et al. 2017; Liu et al. 2018), although labile C, N, and P are usually present together in the natural inputs to soils.

In the current study, we took advantage of gradients in labile C inputs into a temperate forest ecosystem recently documented by Jilková et al. (2020a). Such gradients arise through the long-term effects of wood ant foraging on the inputs of honeydew (sugar-rich secretions of aphids and other insects) to soil. Because the intensity of ant foraging decreases with distance from the ant nest, the inputs of honeydew to soil increase with distance from the ant nest. We collected soils from the surface mineral horizon (A) and the subsoil mineral horizon (B) at 4 m and 70 m from each of four nests. The SOC contents and magnitude of long-term labile C input for the four soil samples are summarized in a previous study (Jilková et al. 2020a). Then, we used the four soils in a laboratory microcosm experiment where we assessed microbial activity (i.e., soil respiration) and PE as affected by the fortnightly addition of labile C alone or in combination with N and/or P (Fig. 1). We tested three hypotheses: (1) the addition of C will result in a higher microbial activity and PE in soil collected from an area with low labile C input (i.e., at 4 m from

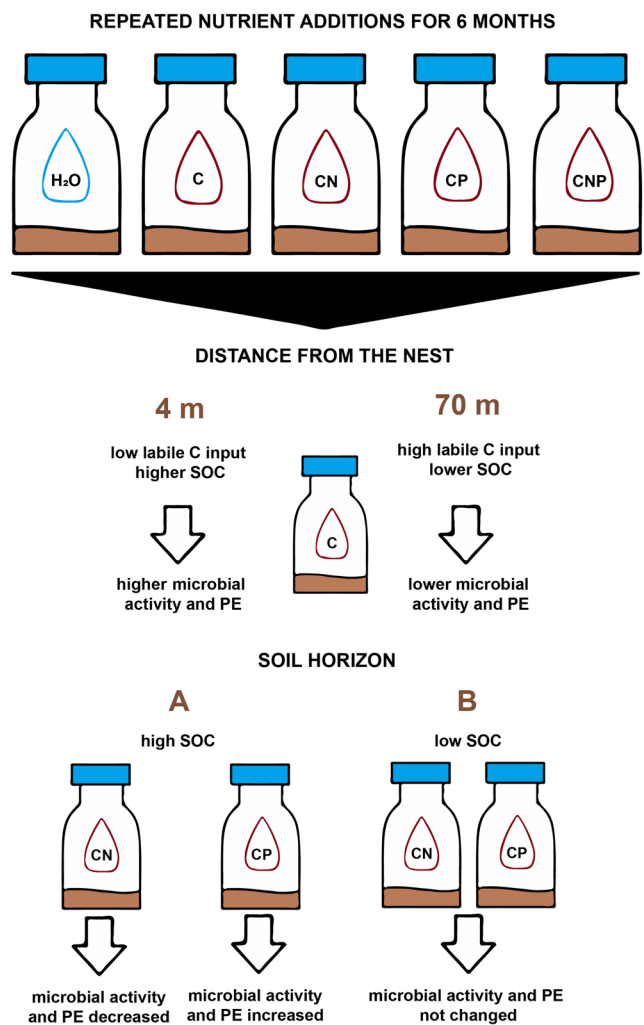


Fig. 1 Conceptual model of the study design and hypotheses (H1-H3)

the nest) than from an area with high labile C input (i.e., at 70 m from the nest); (2) microbial activity and PE in the A horizon (high-SOC soil) will be decreased by N addition because added N will act as a source and thus will attenuate the need for N-mining, but will be increased by P addition because P is not linked to SOM decomposition; and (3) nutrient additions will not affect the microbial activity or the PE in the B horizon because C is the limiting nutrient in low-SOC soils. Testing these hypotheses would advance our understanding of SOC decomposition in temperate coniferous forest soils by revealing the mechanisms by which microbial activity and PE are affected by ecologically relevant nutrient additions and by changes in SOC content connected with changes in labile C inputs.

Materials and methods

Collection of soil samples

Soil samples were collected in a coniferous temperate forest on the southern slope of Klet' Mountain in South Bohemia (Czech Republic) at 800 m a.s.l. (48° 50' 46" N, 14° 18' 1" E) in July 2018. The forest stand consisted entirely of Norway spruce (*Picea abies* (L.) H. Karst.), and soils were Cambisols, derived from a deep colluvial deposit on acid metamorphic rocks (granulite, gneiss), with a thick organic horizon. For a more detailed description of the site and soil profile, see Jílková et al. (2020a). Soil was sampled at horizontal distances of 4 m and 70 m from four wood ant (*Formica* s. str.) nests in an area of 1 ha. As noted in the "Introduction," these two distances differ in the quantity of labile C input in throughfall (in the form of dissolved organic C (DOC)) to the forest floor because of differences in ant foraging activity and hence C-rich honeydew content; i.e., the DOC input is 1.5 times lower at 4 m than at 70 m from the nest (6379 and 9215 mg C m⁻² year⁻¹, respectively; Jílková et al. 2020a).

Soil was sampled from two mineral soil horizons. The A horizon represented the surface mineral soil (ca. 0–10 cm), and the B horizon represented the mineral subsoil (samples taken at 50–60 cm depth). Samples were collected with soil core samplers (46 mm diameter) inserted vertically from the forest floor surface into the A horizon or from the bottom of a trench (50 × 50 × 50 cm) into the B horizon. For each of four ant nests, 3 cores were taken and all 12 core samples homogenized to form one composite sample for each distance and horizon. The 4 soil samples (2 depths × 2 distances) were passed through a 2-mm screen and kept at 4 °C. Soil properties are shown in Table 1. The contents of total organic C (TOC) and total N (TN) in the dried and ball-milled (MM 400, Retsch, Germany; 3 min at 30 Hz) soil samples were determined using a Flash 2000 elemental analyzer (Thermo

Scientific). Total P (TP) was extracted by mineralization in perchloric acid and determined using an ICP-OES. For water-extractable organic C (WEOC), water-extractable N (WEN), and water-extractable P (WEP) determination, samples were placed in deionized water (1:10 soil:water ratio, i.e., 3 g soil:30 mL water) and shaken on a horizontal shaker for 45 min at 80 oscillations per minute at room temperature (Sparling et al. 1998). Suspensions were passed through a filter (Whatman TM, Membrane Filters white, ME 25/21 ST, 0.45 μm), and the filtrate was stored at -20 °C until WEOC and WEN contents were determined with a total organic C analyzer (TOC-L_{CPH} analyzer, Shimadzu) and WEP content was determined by ion chromatography with a Dionex 5000 (Thermo Scientific). Microbial biomass C (*C*_{mic}) was determined using the fumigation-extraction method (Vance et al. 1987). In brief, a 5-g quantity of the fresh soil was placed in 100-mL glass vessels in two replicates. One replicate was immediately processed, whereas the other replicate was subjected to chloroform fumigation for 24 h. The soil from the two replicates was submerged in 40 mL of 0.5 M K₂SO₄, shaken on a horizontal shaker for 45 min at 80 oscillations per min, and passed through a 0.45-μm filter; the filtrate was analyzed for TOC content using a TOC analyzer (model TOC-L_{CPH/CPN}, Shimadzu). *C*_{mic} was calculated as a difference in C content between fumigated and non-fumigated replicate multiplied by the extraction coefficient (0.45).

Incubation experiment

A 50-g quantity of each soil sample was weighed into a 100-mL vessel (microcosm), and bulk density was adjusted to 0.8 g cm⁻³, which was the typical bulk density of this forest site (Jílková et al. 2020a). Vessels were pre-incubated for 2 weeks at 20 °C and were then subjected to one of four nutrient-addition treatments every 2 weeks. The treatments, which were applied in 0.6 mL of deionized water, were as follows: 1.5 mg of C (sucrose) (C), 1.5 mg of C + 0.5 mg of N (NH₄NO₃) (CN), 1.5 mg of C + 0.1 mg of P (NaH₂PO₄·2H₂O) (CP), and 1.5 mg of C + 0.5 mg of N + 0.1 mg of P (CNP). A control without nutrient addition was also included. The C, N, and P amounts correspond to the usual C input to forest soils in throughfall (Jílková et al. 2020a) or to the usual N and P inputs to forest soils in dry and wet depositions (Bradford et al. 2008). The solutions were applied evenly across the soil surface using a pipette. The experimental design resulted in 60 vessels (2 distances × 2 horizons × 4 treatments + the control × 3 replicates). The sucrose originated from a C₄ plant (sugar cane, δ¹³C = -11.88‰), which allowed us to distinguish the proportion of CO₂-C derived from the added C vs. that derived from SOM (δ¹³C = -26.5‰ for the A horizon and δ¹³C = -23.0‰ for the B horizon). Vessels were incubated at 20 °C for 26 weeks.

Table 1 Contents of total organic C (TOC), total N (TN), total P (TP), water-extractable organic C (WEOC), water-extractable N (WEN), water-extractable P (WEP), and microbial biomass C (C_{mic}) in soils collected at two distances from the nest (4 m and 70 m) from two soil horizons (A and B). Values are means \pm SEM ($n = 3$). Within each distance, differences between the soil horizons were significant for all soil properties ($P < 0.05$, t test). Within each soil horizon, different letters indicate significant differences between distances ($P < 0.05$, t test)

Distance (m)	Soil horizon	TOC (mg g ⁻¹)	TN (mg g ⁻¹)	TP (mg g ⁻¹)	WEOC (μ g g ⁻¹)	WEN (μ g g ⁻¹)	WEP (μ g g ⁻¹)	C_{mic} (μ g g ⁻¹)	C_{mic} (mg g ⁻¹ C)
4	A	87.3 \pm 6.9	3.51 \pm 0.30	0.46 \pm 0.02	699 \pm 59	38 \pm 3	13.5 \pm 3.1	166 \pm 12	1.9 \pm 0.1
	B	22.6 \pm 3.0 a	0.91 \pm 0.13 a	0.53 \pm 0.02 a	211 \pm 24 a	16 \pm 1 a	4.5 \pm 0.1	95 \pm 12 a	4.2 \pm 0.5
70	A	75.6 \pm 6.6	3.13 \pm 0.27	0.43 \pm 0.02	648 \pm 54	37 \pm 2	14.5 \pm 5.9	165 \pm 20	2.2 \pm 0.3
	B	11.3 \pm 1.5 b	0.51 \pm 0.05 b	0.29 \pm 0.02 b	98 \pm 13 b	13 \pm 2 b	4.6 \pm 0.1	59 \pm 15 b	5.2 \pm 1.3

The evaporative losses were matched by addition of deionized water.

Respiration in the vessels (an indicator of microbial activity) was determined every 2 weeks in the first half of the incubation period and every 4 weeks in the second half of the incubation period on the day after the nutrient additions. To assess respiration, vessels were closed tightly with a lid with a rubber septum and were left to incubate. After 18 h, 20-mL gas samples were withdrawn using a syringe through the rubber septum and were stored in 12-mL evacuated vials (Exetainer®, Labco Ltd, UK). The gas samples were analyzed within 24 h with an HP 5890 gas chromatograph. CO₂ concentrations were analyzed with a thermal conductivity detector at 100 °C using helium as the carrier gas. The results were normalized to total organic C (TOC) contents. Within 3 days of sampling, the gas samples were subjected to isotope ratio analysis using GasBench coupled to a ConFlo IV mass spectrometer and a Mat 253 mass spectrometer (all instruments were from Thermo Fisher Scientific, Bremen, Germany).

Partitioning calculations

The proportion of CO₂-C derived from C additions ($P_{SUCROSE}$) was calculated using a mass balance equation:

$$P_{SUCROSE} = ({}^{13}C_{NA} - {}^{13}C_{ADD}) / ({}^{13}C_{NA} - {}^{13}C_{SUCROSE}) \quad (1)$$

where ${}^{13}C_{NA}$ is the ‰ value of soil not receiving C additions, ${}^{13}C_{ADD}$ is the ‰ value of soil receiving C additions, and ${}^{13}C_{SUCROSE}$ is the ‰ value of sucrose. Relative priming of SOM decomposition (SOM_{PRIME}) was calculated as:

$$SOM_{PRIME} = R_{ADD}(1 - P_{SUCROSE}) / R_{NA} \quad (2)$$

where R_{ADD} is the cumulative respiration from soil receiving C additions, $P_{SUCROSE}$ is derived from Eq. (1), and R_{NA} is the cumulative respiration from soil not receiving C additions.

Statistical analyses

The effect of distance and soil horizon on soil properties was tested using t test. The effect of soil horizon, distance from the nest, and nutrient-addition treatment on total respiration during the incubation was tested using repeated measures ANOVA ($n = 3$, $df = 40$). The effect of soil horizon, distance from the nest, and nutrient-addition treatment on soil- and substrate-derived respiration and the PE averaged across 6 months of incubation was tested using three-way ANOVAs ($n = 3$, $df = 40$). When tests indicated significant differences, Fisher post hoc tests were used to compare means. All dependent variables were log-transformed to satisfy the assumptions of normality and homoscedasticity for statistical tests.

Statistica 13 software (StatSoft Inc., USA) was used for statistical analyses.

Results

During the whole incubation, total respiration in the control treatment was higher at 4 m than at 70 m and was higher in the A than in the B horizon (Table 2). Total respiration was significantly increased by nutrient-addition treatments (C, CN, CP, and CNP; throughout the whole text, these four treatments but not the control are referred to as the nutrient-addition treatments) relative to the control, and the increase was greater at 70 m than at 4 m and was greater in the B horizon than in the A horizon. Nutrient addition (averaged across the four treatments and relative to the control) increased total respiration by 1.84-fold at 4 m in the A horizon, by 2.21-fold at 70 m in the A horizon, by 4.34-fold at 4 m in the B horizon, and by 10.99-fold at 70 m in the B horizon. However, the effect of the four specific nutrient-addition treatments differed depending on distance and soil horizon. With respect to distance, total respiration at 4 m was lowest in the CN treatment, whereas total respiration at 70 m was highest in the CP treatment. With respect to soil horizon, total respiration did not significantly differ among the four nutrient-addition treatments in the B horizon, but was highest with the CP and CNP treatments in the A horizon.

Substrate-derived respiration averaged across 6 months of incubation was higher at 70 m than at 4 m and was higher in the B horizon than in the A horizon (Fig. 2; Table 2). Substrate-derived respiration was not significantly affected by the four nutrient-addition treatments in the B horizon, but was significantly increased in the CN, CP, and CNP treatments in the A horizon. Soil-derived respiration averaged across 6 months of incubation followed similar trends as total respiration (Fig. 2; Table 2). The increases in soil-derived

respiration after nutrient additions were higher in the B horizon than in the A horizon. Soil-derived respiration did not significantly differ among the four nutrient-addition treatments in the B horizon, but was higher in the CP treatment than in the other nutrient-addition treatments in the A horizon. Soil-derived respiration in the four nutrient-addition treatments did not significantly differ at 4 vs. 70 m in the A horizon but was greater at 70 m than at 4 m in the B horizon. The PE was positive in all cases, and was higher at 70 m than at 4 m and was higher in the B horizon than in the A horizon (Fig. 2; Table 2). The PE did not significantly differ among the four nutrient-addition treatments in the B horizon but was reduced by the CN treatment and increased by the CP treatment in the A horizon.

Discussion

As indicated by respiration, basal microbial activity (i.e., activity in the control treatment) in the current study was higher in the A horizon than in the B horizon and was higher at 4 m than at 70 m from the nest. These differences are likely related to SOC content, which is much higher in the surface (A) mineral horizon than in the sub-soil (B) horizon (Jobbágy and Jackson 2000; Jílková et al. 2020a, b) and which is also higher at 4 m than at 70 m from wood ant nests in the B horizon (Jílková et al. 2020a, b). The differences largely correspond to microbial biomass C expressed per gram soil (linear regression, $R^2=0.41$, $P<0.01$; Table 1). However, although the quantity of SOC clearly regulates microbial activity, the differences in microbial activity cannot be explained simply by microbial biomass C, because we normalized both microbial activity and biomass to SOC content. In fact, microbial biomass was two times higher in the B horizon than in the A horizon when expressed per gram C

Table 2 Results of repeated measures ANOVA and three-way ANOVAs for effects of distance from ant nest (4 and 70 m), soil horizon (A and B), nutrient additions (control, C, CN, CP, CNP), and their interactions on total respiration, soil-derived respiration, substrate-derived

respiration, and the priming effect. *F* values are shown, and *, **, and *** indicate significance at $P<0.05$, <0.01 , and <0.001 , respectively; NS indicates $P>0.05$

Source of variation	Total respiration	Soil-derived respiration	Substrate-derived respiration	Priming effect
Time	2.0 NS	-	-	-
Distance	5.0*	0.1 NS	118.0***	394.0***
Horizon	3201.0***	594.8***	5983.0***	2365.0***
Addition	1308.0***	169.7***	9.0**	9.0***
Distance × horizon	134.0***	38.4***	82.0***	103.0***
Distance × addition	48.0***	19.1***	1.0 NS	1.0 NS
Horizon × addition	299.0***	73.4***	5.0**	4.0*
Distance × horizon × addition	22.0***	12.3***	2.0 NS	1.0 NS

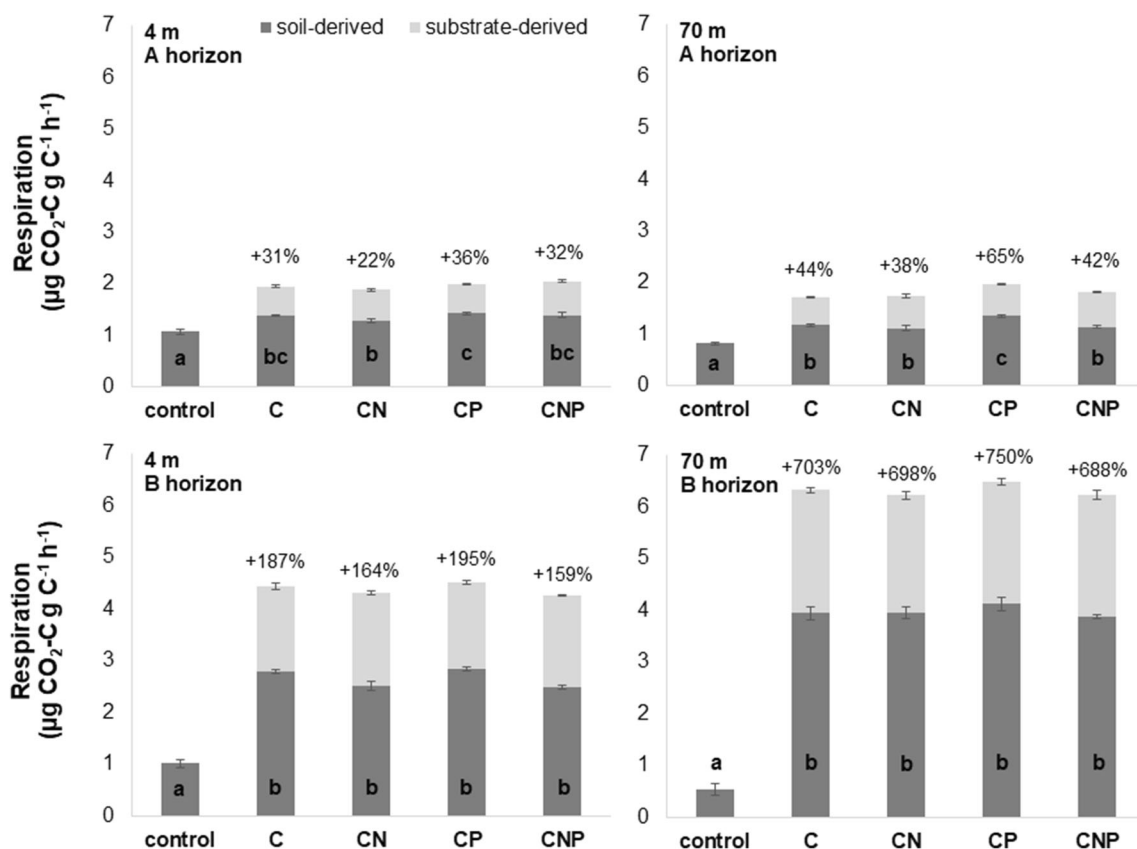


Fig. 2 Soil-derived and substrate-derived respiration averaged across 6 months of incubation as affected by no nutrient addition (control) and nutrient-addition treatments (C, CN, CP, CNP). Values are means \pm SEM

($n = 3$). Different letters indicate significant differences ($P < 0.05$) among the treatments. Percentages refer to the priming effect

(Table 1). The differences might reflect differences in microbial community composition, which can change depending on the differences in SOC stocks (Kramer and Gleixner 2008); i.e., the microbial community closer to the nest might have been better adapted to utilize more complex substrates than the microbial community further from the nest.

An increase in microbial activity following addition of C to soil has routinely been documented in other studies (e.g., Chen et al. 2014; Heitkötter et al. 2017a). However, most previous studies used single additions of a high amount of labile C, which is not realistic and which was previously found to induce a much greater PE than repeated additions of a low amount of labile C (Wang et al. 2019). In the current study using multiple additions of ecologically relevant C amounts, we found a higher microbial activity and PE after C addition at 70 m than at 4 m in both horizons which was inconsistent with our first hypothesis, which was that increases in microbial activity and the PE after C addition would be greater in an area with low labile C input (i.e., at 4 m from the nest) than in an area with high labile C input (i.e., at 70 m from the nest). Perhaps the microbial community had not adapted to the substantial input of labile C at 70 m, i.e., had not experienced an increase in the abundance of microbes better able to utilize

labile C inputs than more complex SOC, which has been previously suggested to occur when labile C inputs are substantial (Fontaine et al. 2003; Paterson et al. 2009; Guenet et al. 2010). However, a more likely explanation is that the microbial community at 70 m had adapted to the low-SOC stock (Jílková et al. 2020b) such that even a low input of labile C during the experiment increased its activity and caused a higher PE similar to the situation in the B horizon. Similar outcomes have been reported by Qiao et al. (2019) who found a higher microbial activity and PE in a young secondary forest with a low-SOC content compared to an old-growth forest with a high-SOC content. In other words, microbial activity and the PE are affected more by the SOC stock than by the magnitude of long-term labile C input.

Most previous studies examined SOC decomposition after additions of separate nutrients (e.g., Wild et al. 2017; Liu et al. 2018), although labile C, N, and P are usually present together in the natural inputs to soils. In the current study, microbial activity and the PE were almost 40% lower after the addition of CN than after the addition of C alone to the A horizon, which was consistent with our second hypothesis. Similarly, Tian et al. (2016) observed that additions of glucose in combination with N reduced the PE by 45% compared to addition of glucose alone. Because of their high C:N ratios, surface

mineral soils are generally N-limited (Brady and Weil 2002). Thus, microbial communities may utilize N compounds added to these soils instead of expending energy to gain N from SOM decomposition; as a result, addition of N to surface soils generally decreases SOC decomposition and the PE (Blagodatskaya et al. 2007; Heitkötter et al. 2017a; Wang et al. 2019). Although Drake et al. (2013) observed a stronger PE after the combined addition of CN compared to the addition of C alone to a temperate forest mineral soil, the C:N ratio of the CN addition was 10 in that study but was only 3 in the current study. The higher C:N ratio apparently did not alleviate N limitation such that the microbial community used the added substrate to decompose SOM, and the PE increased as a consequence. The increased microbial activity and PE caused by P addition to the A horizon in the current study was consistent with our second hypothesis. Similar results were also found in previous studies focusing on P additions into nutrient-limited tropical forest soils (Cleveland and Townsend 2006; Cleveland et al. 2006; Gnankambary et al. 2008). Because P is a limiting nutrient in coniferous forest ecosystems (Batjes 1997), which is supported by a high C:P ratio (183 on average) in the A horizon in the current study, it is reasonable that P addition increased microbial activity and therefore SOM decomposition by alleviating the P limitation. On the other hand, the combined addition of CNP caused no change in microbial activity or the PE compared to the addition of C alone. N and P additions apparently had equal but opposite effects.

Microbial activity and the PE in the B horizon were increased by the addition of C alone but were not further increased or decreased by addition of CN, CP, or CNP, which was consistent with our third hypothesis. Similar findings were reported in the surface mineral soil of a subtropical forest (Qiao et al. 2016) as well as in the mineral subsoil of a deciduous temperate forest (Heitkötter et al. 2017b). Previous studies suggested that SOC turnover in the subsoil is largely limited by the labile C input (Zhang et al. 2015; Karhu et al. 2016; Heitkötter et al. 2017a, b; Jia et al. 2017). In addition, the C:N ratio is low in the subsoils (Rumpel and Kögel-Knabner 2011), which might further increase C limitation of the microbial community. Although such a situation occurred in the deciduous forest studied by Heitkötter et al. (2017b), the C:N ratio of the subsoil of that forest was 10.5, whereas the C:N ratio of the coniferous forest subsoil in the current study was 22–25, i.e., C may have been more available in the subsoil of the current study than in the subsoil investigated by Heitkötter et al. (2017b). Nevertheless, because a large proportion of the subsoil SOC originates as litter leachates (Rumpel and Kögel-Knabner 2011), which are represented mainly by fulvic acids and phenolics in coniferous forests (Berg and McLaugherty 2008), SOC might be less available and thus more limiting in coniferous forests than in deciduous forests such that the addition of labile C may cause similar

increases in microbial activity in the subsoils of both kinds of forests.

Conclusions

We found that the increases in PE caused by addition of labile C were greater in soils with low levels of SOC (i.e., in subsoils or in soils with high long-term labile C inputs) than in soils with high levels of SOC, suggesting that increases in labile C inputs to soils might greatly affect not only deep low-SOC soils but also soils where SOC had been already depleted by increased labile C input. Although increased N and P additions to deep mineral soils did not affect microbial activity beyond the effect of additions of C alone, the addition of N to surface mineral soils suppressed SOC mineralization, such that N deposition might lead to higher SOC storage in surface mineral soils. However, future research is needed to explain if the differences between low-SOC and high-SOC soils are caused by changes in microbial biomass or by changes in microbial community composition and/or enzymatic activity.

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Data availability Not applicable.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare no competing interests.

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