Biology and Fertility of Soils (2020) 56:411–421
https://doi.org/10.1007/s00374-020-01433-4

ORIGINAL PAPER

Organic matter decomposition and carbon content in soil fractions as affected by a gradient of labile carbon input to a temperate forest soil

Veronika Jílková[1] · Kateřina Jandová[2] · Tomáš Cajthaml[2,3] · Miloslav Devetter[1,4] · Jaroslav Kukla[2] · Josef Starý[1] · Anna Vacířová[2]

Received: 29 August 2019 / Revised: 13 January 2020 / Accepted: 16 January 2020 / Published online: 20 January 2020

Abstract

Labile carbon (C) input to soils is expected to affect soil organic matter (SOM) decomposition and soil organic C (SOC) stocks in temperate coniferous forests. We hypothesized that the SOM decomposition rate, C content in soil fractions, and microbial and faunal abundance and activity were increased along the gradient in labile C input around wood ant nests. Three distances from the nest that differed in annual labile C input to soil were selected: 4 m with 6379 mg C m^{-2}, 30 m with 9060 mg C m^{-2}, and 70 m with 9215 mg C m^{-2}. Soil from the organic horizon (Oe+Oa), surface mineral horizon (A), and subsoil mineral horizon (B) was analyzed for C content in soil fractions and for activity and abundance of soil microorganisms and fauna. In addition, a 1-year litter-bag and soil-bag decomposition experiment was conducted. Although the rate of soil decomposition did not differ along the labile C input gradient, the rate of litter decomposition in the B horizon increased as labile C input increased with distance from the nest. Correspondingly, the C content in bulk soil and in the labile and less-protected soil fractions in the B horizon decreased as labile C input increased. We infer that, because the O and A horizons are less C-limited than the B horizon, the changes in the labile C input along the gradient affected the B horizon more than the surface O and A horizons. However, soil microbial and faunal activity and abundances were not consistently affected by the gradient. Apparently, C stocks in soil fractions are more important for microbial and faunal communities than labile C inputs. Although the results indicate that SOC content changes very slowly in the coniferous forest soil of the current study, increases in the input of natural labile C leads to decreases in the SOC stock in the B horizon. By increasing the labile C input to temperate forest soils, future increases in atmospheric CO_2 concentration may therefore lead to a significant loss of SOC in deep soil layers.

Keywords Coniferous forest · Litter bag · Microbial activity · PLFA · Soil fauna

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00374-020-01433-4) contains supplementary material, which is available to authorized users.

✉ Veronika Jílková
veronika.jilkova@upb.cas.cz

[1] Biology Centre, Institute of Soil Biology and SoWa RI, Czech Academy of Sciences, Na Sádkách 7, CZ-37005 České Budějovice, Czech Republic

[2] Institute for Environmental Studies and SoWa RI, Faculty of Science, Charles University, Benátská 2, CZ-12801 Prague, Czech Republic

[3] Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ-14220 Prague, Czech Republic

[4] Centre for Polar Ecology, Faculty of Science, University of South Bohemia, Na Zlaté stoce 3, CZ-37005 České Budějovice, Czech Republic

Introduction

Forest ecosystems contain a large part of the carbon (C) stored on land, and this terrestrial C is stored as both biomass and soil organic matter (SOM) (Hyvönen et al. 2007). Because C stocks in northern temperate forests are two times greater in the soil than in the vegetation (Dixon et al. 1994; Schlesinger 1997), changes in soil C stocks can be more important than changes in vegetation C stocks for the C budgets of these forests (Medlyn et al. 2005). As a consequence, substantial research has been directed at understanding the C budget of temperate forest soils as influenced by the balance between SOC decomposition and sequestration (e.g., Bradford et al. 2008; Hyvönen et al. 2007; Melillo et al. 2011; Wiesmeier et al. 2013). Both of these processes might be greatly affected by the availability of labile C (Gunina and Kuzyakov 2015;

Qiao et al. 2014), which could increase with increases in atmospheric CO_2 concentrations and the resulting increases in primary production (Ceulemans et al. 1999; Norby et al. 1999). It follows that additional research is needed on the effects of labile C inputs on the forest soil C budget.

Soils contain a broad spectrum of C structures, which range from fresh labile C, such as plant litter leachates or root exudates, to more recalcitrant SOC, which refers to SOM no longer recognizable as plant litter (Blagodatskaya and Kuzyakov 2008). Recalcitrant SOC might be greatly affected by the input of labile C, which changes the SOM decomposition rate via the priming effect (Kuzyakov 2010; Kuzyakov et al. 2000). Such a situation has already been observed in broadleaf forests (Hopkins et al. 2014; Wang et al. 2015) as well as in coniferous forests (Crow et al. 2009; Ganjegunte et al. 2006; Sulzman et al. 2005). Although priming may mineralize some recalcitrant SOC and lead to the loss of C via CO_2, excessive inputs of labile C to soil may also transfer into stabilized pools of SOC such that the loss of C might be buffered by increased SOC sequestration (Hyvönen et al. 2007; Wang et al. 2015). These processes might be particularly important for subsoil C pools, which contain a substantial portion of the stored SOC (Jobbágy and Jackson 2000) and which is more susceptible to changes in labile C input because it is more C-limited than surface mineral soils (Wang et al. 2014). In addition, low C quality and availability in subsoil mineral horizons might also limit microbial decomposition of SOM (Baldock and Skjemstad 2000). However, such inferences have not been confirmed for temperate coniferous forest soils.

Although labile C undergoes various processes with passage through the soil profile including sorption to soil particles, aggregate formation, or leaching (Gunina and Kuzyakov 2015), uptake and transformation by microorganisms are the most significant (Biernath et al. 2008; Fisher et al. 2010). Bacteria are generally better able than fungi to utilize labile C (Koranda et al. 2014), but coniferous forest soils with low pH favor the activity of soil fungi, which are more tolerant than bacteria to low pH (Brady and Weil 2002). Therefore, fungi are expected to be abundant in coniferous forest soils and to play a more important role in labile C processing. Like soil microbes, saprotrophic soil fauna feed on root exudates and other forms of labile C (Ruf et al. 2006; Scheu 2001). In addition, soil fauna may indirectly benefit from labile C when feeding on fungi and bacteria that acquired labile C (Albers et al. 2006). Thus, the soil fauna community is expected to be positively affected by labile C inputs.

Most previous studies on the effect of increased labile C inputs to soil used artificially increased C inputs in the form of simple sugars (e.g., Bradford et al. 2008; Chigineva et al. 2009; Haohao et al. 2017; Hopkins et al. 2014; Nieminen and Pohjola 2014; Qiao et al. 2014). However, sugars poorly represent labile C because plant-derived labile C also contains organic acids, amino acids, and phenolics (Bertin et al. 2003; Isidorov et al. 2010; Kalbitz et al. 2006). An alternative way of assessing the effects of increased labile C inputs could be the use of the naturally occurring labile C gradients in the surroundings of wood ant nests (Jílková et al. 2020). Such gradients arise when ant foraging depletes honeydew inputs to soils near ant nests. These gradients can be steep. In the study by Jílková et al. (2020), for example, the difference between the lowest C input (near the nest, highly affected by ants) and the highest C input (> 70 m from the nest, not affected by ants) was 1.5-fold. Given that photosynthesis is expected to increase by 60% by the end of this century due to climate change (Norby et al. 1999), we considered that information on the effects of the spatial gradient in labile C inputs around ant nests could be useful for understanding the effects of the predicted temporal gradient in labile C inputs to forest soils resulting from climate change.

In the present study, we determined how SOM decomposition, C content in soil fractions, and microbial and faunal abundance and activity are affected by the gradient in labile C input around wood ant nests. We tested three hypotheses: (1) both SOM decomposition and C content in soil fractions will increase as labile C input increases with distance from ant nests, (2) greater changes will occur in the subsoil mineral horizon than in the surface horizons due to higher C limitation in the subsoil, and (3) the abundances of soil fungi and fauna will increase as labile C input increases with distance from ant nests.

Materials and methods

Study site and sampling transects

The study was carried out in a coniferous temperate forest on the southern slope of Kleť Mountain in South Bohemia (Czech Republic) at 800 m.a.s.l (48° 50′ 46″ N, 14° 18′ 1″ E). The forest stand consisted entirely of Norway spruce (*Picea abies* (L.) H. Karst.) with a mean tree age of 70 years and a dominant tree height of 30 m. Based on long-term measurements (2005–2015), the mean annual temperature was 7.12 °C and the mean annual precipitation was 720 mm (data from the Kleť observatory and the nature reserve area Blanský les). The area had Cambisols derived from a deep colluvial deposit on acid metamorphic rocks (granulite, gneiss) with a thick organic horizon. The soil at the study site had a bulk density of 0.30 ± 0.02 (mean ± SE), 0.67 ± 0.03, and 1.20 ± 0.03 g cm^{-3} in the O, A, and B horizon, respectively. The pH was 4.00 ± 0.04, 4.12 ± 0.04, and 4.85 ± 0.04 in the O, A, and B horizon, respectively. The OM content was 0.56 ± 0.03, 0.13 ± 0.01, and 0.05 ± 0.01 g g^{-1} in the O, A, and B horizon, respectively. For a more detailed description of the soil profile, see Jílková et al. (2020).

Several thousand wood ant (*Formica* s. str.) nests are distributed over the southern slope of Kleť Mountain (Miles 2000). We selected four medium-sized nests (ca. 1 m^3) distributed over an area of 1 ha. A sampling transect was established around each nest as described by Jílková et al. (2020). Each transect was 70 m long with sampling locations at 4 m, 30 m, and 70 m. Based on Jílková et al. (2020), the annual labile C input was 6379 mg C m^{-2} at 4 m, 9060 mg C m^{-2} at 30 m, and 9215 mg C m^{-2} at 70 m; the area > 60 m from the ant nests was not affected by ant foraging activity. This sampling design yielded a total of 12 sampling locations (4 nests × 3 distances).

Soil sampling and processing

Soil was sampled three times throughout the vegetative season (i.e., in April, July, and October 2017) from three horizons (O, A, and B), giving a total of 108 samples. The O horizon was the organic horizon which varied in thickness and consisted of moderately to highly decomposed organic matter (Oe+Oa according to the USDA soil taxonomy). The A horizon represented the surface mineral soil (ca. 0–10 cm depth), and the B horizon represented the mineral subsoil (ca. 50–60-cm depth). Samples for analysis of microbial properties and physical fractionation of SOC were collected by inserting soil core samplers (46-mm diameter) vertically from the forest floor surface into the O and A horizon or from the bottom of a trench (50 × 50 × 50 cm) into the B horizon; a trench was dug at each sampling location. Samples were taken in three replicates ca. 10 cm from each other that were pooled to form one composite sample per sampling location and horizon. Then, they were passed through 2-mm screens and air-dried (for physical fractionation), kept at 4 °C (for assessment of soil respiration), or freeze-dried (for analysis of phospholipid fatty acids, PLFAs). Additional samples were collected with soil core samplers (36 mm diameter) and were kept at 4 °C for mesofauna and microfauna extraction.

Physical fractionation of SOC

Physical fractionation was carried out for samples collected in July from the A and B mineral horizons. The procedure described by Six et al. (1998, 2000) was used to determine C storage in six SOC fractions. In brief, 30 g of a soil sample were placed in 150 mL of dH_2O together with 50 glass beads. The suspension was shaken on a horizontal shaker at 80 oscillations per minute for 15 min and was then wet-sieved through a 250-μm screen and then through a 53-μm screen. Coarse particulate OM (the cPOM fraction) was collected on the 250-μm screen. The material that passed through the 53-μm screen was centrifuged at 3200 g for 30 min, such that the pellet contained silt and clay in macroaggregates (the S+C_{MAC} fraction). A 5-g sample of the material that passed through the 250-μm screen and that was collected on the 53-μm screen was subjected to density fractionation using sodium polythungstate (SPT) at a density of 1.8 g cm^{-3}. The sample was placed in 35 mL of SPT and centrifuged at 1370 g for 10 min, such that the supernatant contained the light (< 1.8 g cm^{-3}) intra-macroaggregate POM fraction (the POM_{MAC} fraction). The pellet (> 1.8 g cm^{-3}) was placed in 30 mL of dH_2O with 12 glass beads, shaken overnight on a horizontal shaker at 80 oscillations per minute, and wet-sieved through a 53-μm screen. The material collected on the 53-μm screen represented intra-microaggregate POM (the POM_{MIC} fraction). The material that passed through the 53-μm screen was centrifuged at 3200 g for 30 min, such that the pellet contained silt and clay in microaggregates (the S+C_{MIC} fraction). All of six fractions were dried at 40 °C to a constant weight, ball-milled, and analyzed for total organic C (TOC) using a TOC-$L_{CPH/CPN}$ model TOC analyzer coupled with an SSM-5000A solid sample module (Shimadzu). To assess dissolved organic C (DOC), 1.5 g of soil were placed in 50 mL of dH_2O with 6 glass beads; the suspension was shaken on a horizontal shaker for 15 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).

Microbial properties

Microbial properties were analyzed in samples from all three horizons. Soil respiration was analyzed several days after sampling. A 10-g quantity of the fresh soil was placed in 100-mL glass vessels. Three vessels were left empty to determine the CO_2 concentration of the air (blank). The vessels were pre-incubated for 24 h at room temperature. A beaker with 3 mL of 1 M NaOH was then placed in each vessel and vessels were incubated for 3 days at room temperature before respiration was quantified by titration of the NaOH according to Page (1982).

PLFAs were extracted from freeze-dried soil with a chloroform-methanol-phosphate buffer (1:2:0.8) (Bligh and Dyer 1959). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60, Merck), and the samples were subjected to mild-alkaline methanolysis. The free methyl esters of PLFAs were analyzed by gas chromatography-mass spectrometry (450-GC, 240-MS ion trap detector, Varian, Walnut Creek, CA, USA) as previously described (Jílková et al. 2015).

In total, 20 PLFA compounds were consistently detected above the thresholds required for accurate quantification and were used to assess the composition of main microbial groups. PLFAs were assigned to indicator groups as follows: fungi 18:2ω6,9, 18:1ω9 (Klamer and Bååth 2004); actinobacteria 10Me-16:0, 10Me-17:0, 10Me-18:0 (Hanson et al. 1999; Zelles 1999); Gram-positive (G^+) bacteria i15:0, a15:0, i16:0, i17:0, a17:0 (Hanson et al. 1999; Butler et al. 2003);

and Gram-negative (G⁻) bacteria 16:1ω7, 18:1ω7, cy17:0, cy19:0 (Hanson et al. 1999; Zelles 1999). PLFA compounds that were not attributable to specific groups were included for multivariate analyses of microbial community composition. Microbial biomass C (C_{mic}) was calculated as the sum of all fatty acid esters in nanomole multiplied by a conversion factor of 2.4 based on the assumption that 1 nmol of PLFA corresponds to 2.4 μg of C (Bailey et al. 2002).

Fauna extraction and analysis

Fauna abundances were analyzed in samples from the O and A horizon only. Mesofauna were extracted using a high-efficiency "high-gradient" photo-thermo-extractor modified according to Marshall (1972). Heat extraction was performed over 5 days at increasing temperatures of 25, 27, 30, 33, and 40 °C. The average efficiency of the extractor used at these temperatures was experimentally determined to be 93.5% for adult stages of mesofauna. A picric acid-saturated water solution was used as a fixation medium. The extracted mesofauna were filtered out of the picric acid solution and transferred to 80% denatured ethanol in Eppendorf tubes. Individuals were cleared in an 80% solution of lactic acid in water before they were examined. Microfauna were extracted from soil into distilled water using an L-C extractor (Devetter 2010). Soil fauna samples were processed in a random order as soon as possible, but no later than 1 month after collection to prevent community changes. Counting and sorting were performed with a stereomicroscope for mesofauna or an inverted microscope for microfauna.

Decomposition experiment

Litter bags and soil bags were used to assess the decomposability of litter and soil along the gradient. Litter subsamples were collected in April 2017 from the litter layer (Oi) at each sampling location and were combined to form one composite litter sample. Soil was collected in April 2017 at each sampling location from each horizon (Oe+Oa, A, B); the soil samples were not combined into a composite sample. Litter and soil were air-dried. Litter was then placed in thin layers in plastic bags and γ-ray sterilized (isotope Co^{60}, total dose 40 kGy) as described in Frouz and Nováková (2002) so that litter decomposition resulted from the activity of the organisms surrounding the litter bags rather than in the litter. A part of the litter might have transformed to dissolved OM due to lysis of microbial as well as plant cells during the sterilization process which might have relatively increased the rate of decomposition. Nonetheless, since the litter placed into all litter bags derived from the same composite sample and the amount of dissolved OM was the same, the comparison of treatments was not affected. All of the bags used for assessing decomposition were constructed of mesh and were 10 × 10 cm in size, but litter bags had 1-mm openings while soil bags had 42-μm openings. Litter bags, each containing 7 g of sterilized litter, were placed at each sampling location and horizon. Soil bags, each containing 15 g of non-sterilized soil from the O horizon or 50 g of non-sterilized soil from the A or B horizons, were placed in the location where the soil was collected. The soil bags contained more material than the litter bags because the OM content was substantially lower in soil than litter, and detecting OM mass loss would therefore require a greater quantity of soil than litter. The bags were placed in the field in July 2017. After the bags were collected in July 2018, the material in each bag was air-dried and mass loss was calculated.

Statistical analyses

Data were analyzed using mixed linear models with distance, horizon, and season as fixed factors, and ant nest as a random factor. Dependent variables were log-transformed before analysis when needed to improve the normality of residuals. The highest-level interactions were never significant and were therefore excluded from the models. When tests indicated significant differences, Fisher LSD post hoc tests were used to compare means. The effect of distance from the ant nest on contents of SOC fractions was determined using partial redundancy analysis (RDA). The effect of ant nest (replications) and soil horizons on total variability was partialled out as covariables. The abundances of main microbial groups (PLFA relative abundance, mol%) and fauna abundance were analyzed by principal component analysis (PCA). Loadings of PLFA compounds and fauna groups on each principal component axis are reported to indicate important sources of variation responsible for discriminating among samples in each analysis. All analyses were performed using Statistica 13 and Canoco 5 software.

Results

SOC fractionation

According to a partial RDA, C content in soil fractions was significantly affected by the distance from the nest (Fig. S1) ($_{pseudo}F = 6.9$, $P < 0.01$, 999 permutations), but the effect of soil horizon was stronger than the effect of distance: distance explained 25.6% and soil horizon explained 70.9% of the data variability. Similar results were obtained with a two-way ANOVA (Table 1). The A horizon generally had a higher C content than the B horizon. Carbon content generally decreased with the distance from the ant nest. This effect, however, was significant only in the B horizon; specifically, significant differences with distance in the B horizon were found

Table 1 Carbon content (mg C g^{-1} soil) in soil fractions and bulk soil as affected by the distance from the nest (4, 30, and 70 m) and soil horizon (A and B). Values are means ± SEs. For each horizon, means with different letters are significantly different ($P < 0.05$). F and P values for mixed linear models are shown. *, **, and *** indicate significance at $P < 0.05$, < 0.01, and < 0.001, respectively; NS indicates $P > 0.05$. Two-way interactions were never significant and were not included in the models

Horizon	Distance	cPOM	POM_{MAC}	$S+C_{MAC}$	POM_{MIC}	$S+C_{MIC}$	DOC	Bulk soil
A	4	19.2 ± 8.2	24.8 ± 7.6	52.8 ± 5.8	0.25 ± 0.06	0.16 ± 0.06	0.81 ± 0.14	98.0 ± 16.3
	30	9.8 ± 2.4	11.7 ± 3.5	42.8 ± 8.3	0.26 ± 0.04	0.28 ± 0.07	0.61 ± 0.12	65.5 ± 13.5
	70	9.7 ± 1.3	12.0 ± 3.1	34.6 ± 4.4	0.33 ± 0.07	0.37 ± 0.12	0.61 ± 0.09	57.6 ± 8.5
B	4	5.2 ± 1.6 a	3.9 ± 1.9 a	13.3 ± 3.8 a	0.20 ± 0.03	0.21 ± 0.03	0.21 ± 0.06 a	23.1 ± 7.2 a
	30	2.0 ± 0.5 ab	1.7 ± 0.5 ab	11.1 ± 2.1 a	0.19 ± 0.03	0.15 ± 0.03	0.11 ± 0.03 ab	15.3 ± 3.0 ab
	70	1.2 ± 0.2 b	1.0 ± 0.3 b	6.5 ± 0.8 b	0.10 ± 0.01	0.12 ± 0.04	0.08 ± 0.01 b	9.0 ± 1.3 b
Source of variance	df	cPOM	POM_{MAC}	$S+C_{MAC}$	POM_{MIC}	$S+C_{MIC}$	DOC	Bulk soil
Distance	2, 11	8.5 **	3.8 *	4.4 *	0.5 NS	0.1 NS	5.2 *	6.6 *
Horizon	1, 11	80.7 ***	61.6 ***	114.5 ***	7.8 *	1.3 NS	144.5 ***	122.7 ***

in bulk soil and in cPOM, POM_{MAC}, $S+C_{MAC}$, and DOC fractions (Table 1).

Soil microorganisms

Means of soil microbial properties and statistical results of mixed-effect linear models as affected by the distance from the nest, season, and soil horizon are presented in Figs. 1 and 2 and Table 2. Soil horizon had the strongest effect, followed by season. Soil respiration, C_{mic}, and fungal relative abundance were highest in the O horizon and decreased with depth. The relative abundances of actinobacteria, G^+, and G^- bacteria, in contrast, were lower in the O horizon than in the mineral soil horizons. Soil respiration was higher in July than in April and October. The relative abundance of microbial groups showed no consistent pattern with respect to season, i.e., the abundance was generally highest in April for G^+ and G^- bacteria but in October for fungi and actinobacteria. The overall effect of distance from the nest was significant only for C_{mic}, which was higher at 4 m than at 30 m and 70 m from the nest. However, the effect of distance was dependent on season; the relative abundance of actinobacteria in July was greater at 4 m than at 30 m and 70 m, but the relative abundance of G^+ bacteria in October was lower at 4 m than at 30 m and 70 m from the nest. Multivariate analysis of PLFA community composition showed that community composition changed with distance in the B horizon in April and July (Fig. S2). The community discriminated similarly on the first (PC1) and second principal component (PC2). The loadings on these axes indicated that the increased relative abundance of actinobacteria (10Me-16:0, 10Me-17:0, 10Me-18:0) and G^+ bacteria (a15:0, i17:0, a17:0) were the strongest factors causing discrimination of the of the main microbial groups at 4 m distance from that at 30 and 70 m (Table S1).

Soil fauna

Means of soil fauna abundances and statistical results of mixed-effect linear models as affected by the distance from the nest, season, and soil horizon are presented in Figs. 3 and 4 and Table 3. Again, soil horizon had the strongest effect, followed by season. Fauna abundances were always higher in the O horizon than in the A horizon. The abundances of microfauna groups were higher in April than in July and October. The abundances of mesofauna groups, in contrast, were lower in April than in July and October. The overall effect of the distance from the nest was significant only for the Oribatida and was marginally significant ($P = 0.08$) for the Prostigmata, with a higher abundance at 4 m than at 30 and 70 m. However, the effect of distance depended on season; in April and October, Prostigmata and Nematoda abundances were higher at 4 m than at 30 m and 70 m from the nest. Multivariate analysis of fauna community composition showed distinct communities in the O horizon vs. the A horizon (Fig. S3). The community discriminated primarily on the first principal component (PC1). The loadings on this axis indicated that the greater abundance of all fauna groups in the O horizon explained the discrimination of the fauna community between the O horizon and the A horizon (Table S2).

Decomposition experiment

Litter mass loss did not differ among soil horizons ($F_{2,18} = 2.27$, $P = 0.13$) but differed with the distance from the nest ($F_{2,18} = 3.81$, $P < 0.05$) (Fig. 5a). Litter mass loss increased with distance from the nest in both the A and B horizon but was statistically significant only in the B horizon. Soil mass loss differed among soil horizons ($F_{2,18} = 134.0$, $P < 0.001$), and was highest in the O horizon, intermediate in the A horizon, and lowest in the B horizon (Fig. 5b). Soil mass loss was not significantly affected by distance from the nest ($F_{2,18} = 0.9$, $P = 0.43$).

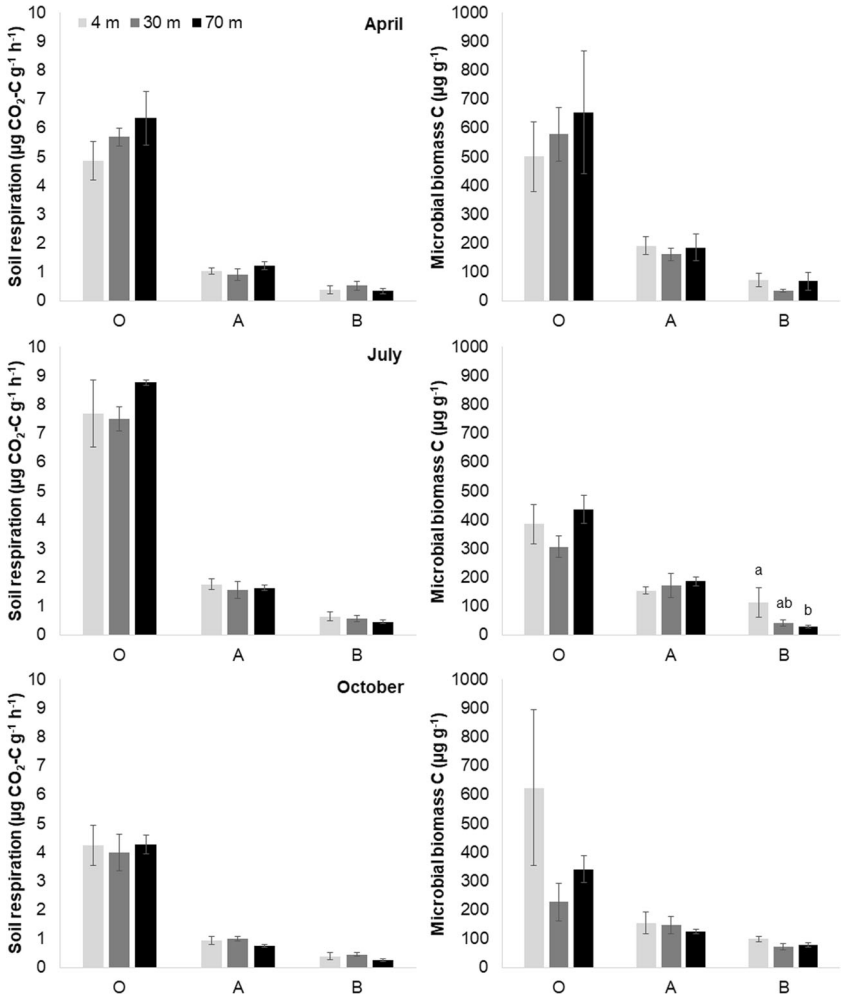

Fig. 1 Soil respiration and microbial biomass C as affected by the distance from the nest (4, 30, and 70 m), soil horizon (O, A, and B), and season (April, July, and October). Values are means ± SEs. For each season and horizon, means with different letters are significantly different ($P < 0.05$)

Discussion

Litter decomposition rates increased as labile C inputs increased, i.e., litter decomposition rates were higher at 30 m and 70 m from wood ant nests than at 4 m from the nests. Our results are in agreement with previous studies which found that labile C inputs to soils increased SOM decomposition rates in coniferous forests (Crow et al. 2009; Ganjegunte et al. 2006; Sulzman et al. 2005). However, although the trend was evident in both the A and B horizon, it was only significant in the B horizon. The B horizon is considered to be C-limited because of the lack of C inputs into the subsoil (Wang et al. 2014) and low C quality and availability (Baldock and Skjemstad 2000). We infer that, because the O and A horizons are less C-limited than the B horizon, the changes in the labile C input along the gradient affected the B horizon more than the surface O and A horizons. These results are consistent with our previous study, which found that SOM contents in the B horizon were higher at 4 m than at greater distances from the nest (Jílková et al. 2020). The results show the effect of the native microbial community on litter decomposition because the litter had been γ-ray sterilized before it was added to the litter bags. Litter decomposition rates did not differ among the soil horizons. However, that suggested a higher capacity of the native microbial communities in deeper soil horizons to decompose newly introduced organic materials, since the microbial biomass decreases considerably with soil depth.

On the other hand, when native soil substrates were used for the decomposition experiment (i.e., the soil-bag experiment), the effect of soil horizon was significant, specifically, the decomposition rate was highest in the O horizon and lowest in the B horizon. This suggests that soils at different horizons differ in their decomposability, i.e., in the SOM content that is available to be decomposed. Although the labile C input into the B horizon may have been affected by distance, the soil decomposition rate (as indicated by mass loss from soil bags) was not significantly affected by distance in any of the three horizons.

The C contents in the bulk soil as well as in soil fractions decreased with distance from the nest. This natural gradient of labile C input in a forest ecosystem represents relatively low inputs that probably stimulated the microbial activity and

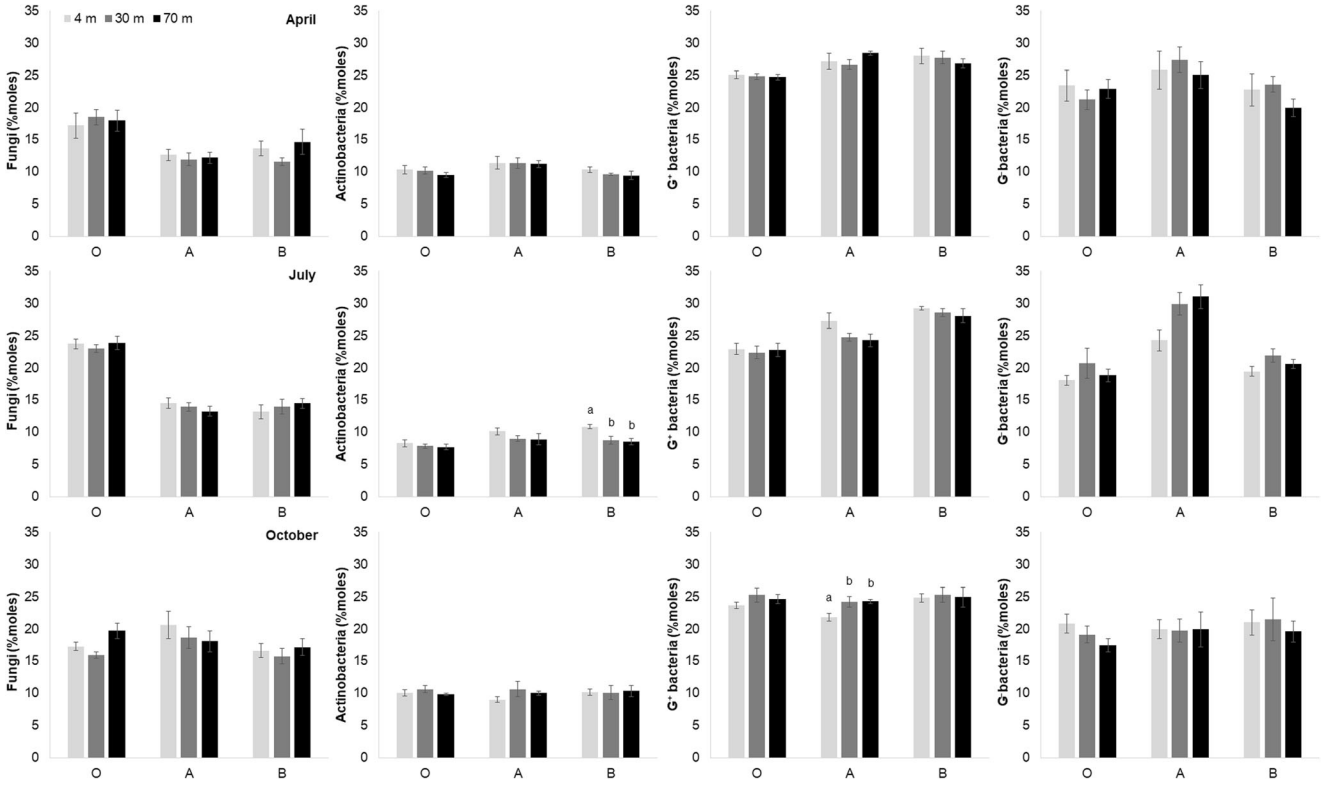

Fig. 2 Relative abundance of microbial groups as affected by the distance from the nest (4, 30, and 70 m), soil horizon (O, A, and B), and season (April, July, and October). Values are means ± SEs. For each season and horizon, means with different letters are significantly different ($P < 0.05$)

therefore led to the loss of SOC stock. Similar conclusions were presented in previous studies that used free-air CO_2 enrichment (FACE) setups (e.g., Hoosbeek et al. 2004). This shows that ecologically relevant increase in labile C input causes contradictory effect to studies that applied excessive C amount inputs, such as the study by Wang et al. 2015, who applied C causing saturation of the microbial community, leading to the stabilization of the excessive labile C and thereby increasing SOC stocks.

Differences in the C content of soil fractions along the gradient were found only in labile C fractions (i.e., coarse POM and DOC, which are not protected by mineral associations) or in less-protected C fractions (i.e., POM occluded by or bound onto mineral particles in macroaggregates), which is not surprising given that non-protected and macroaggregate-associated SOC shows a low level of protection (Six et al. 2002) and therefore is largely susceptible to changes in labile C inputs. On the other hand, microaggregates provide a higher level of protection than macroaggregates and therefore are considered to provide a higher degree of SOC stabilization (Six et al. 2000, 2002).

The effect of the distance on the C content in soil fractions was significant only in the B horizon. This again confirms the assumption that the B horizon is C-limited and that the input of labile C therefore causes larger changes in the B horizon than in the surface horizons. Unlike the C content in soil fractions, however, the soil decomposition rate was not

Table 2 Results of mixed-effect linear models for soil respiration, microbial biomass C (C_{mic}), and relative abundance of microbial groups as affected by the distance from the nest (4, 30, and 70 m), soil horizon (O, A, and B), and season (April, July, and October). *F* and *P* values for are shown. *, **, and *** indicate significance at $P < 0.05$, < 0.01, and < 0.001, respectively; NS indicates $P > 0.05$. Three-way interactions were never significant and were not included in the models

Source of variance	df	Soil respiration	C_{mic}	Fungi	Actinobacteria	G^+ bacteria	G^- bacteria
Season	2, 80	23.5 ***	0.8 NS	21.0 ***	21.8 ***	15.8 ***	12.6 ***
Horizon	2, 80	485.9 ***	143.1 ***	46.2 ***	5.0 **	27.2 ***	15.7 ***
Distance	2, 80	0.4 NS	4.0 *	1.3 NS	2.8 NS	0.03 NS	1.2 NS
Season × horizon	4, 80	0.5 NS	4.5 **	15.0 ***	5.0 **	10.0 ***	6.0 ***
Season × distance	4, 80	1.0 NS	0.3 NS	0.3 NS	3.0 *	2.6 *	1.9 NS
Horizon × distance	4, 80	2.0 NS	1.7 NS	1.2 NS	1.1 NS	0.4 NS	0.8 NS

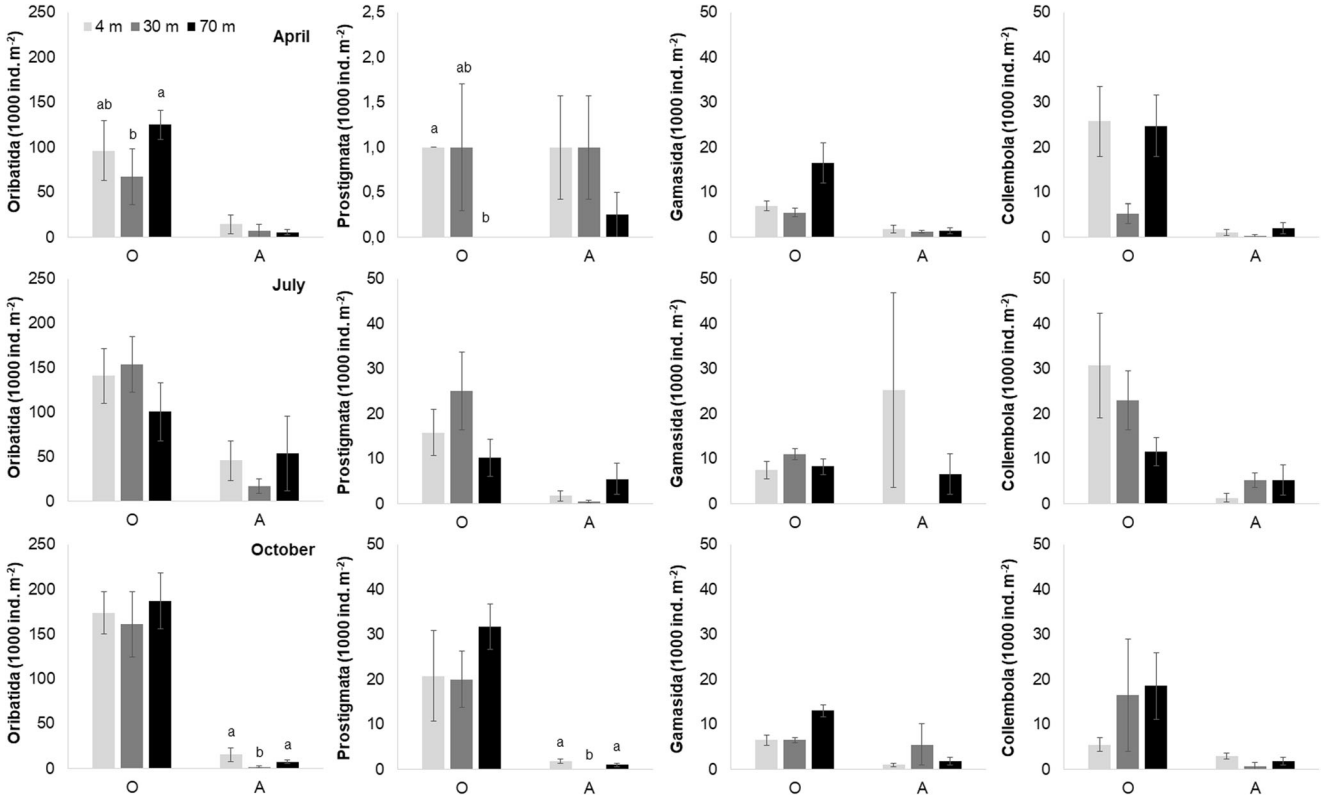

Fig. 3 Numbers of individuals (1000 ind m^{-2}) of mesofauna as affected by the distance from the nest (4, 30, and 70 m), soil horizon (O and A), and season (April, July, October). Values are means ± SE. For each season and horizon, means with different letters are significantly different ($P < 0.05$)

affected by the distance from the ant nest in the B horizon. Apparently, the 1-year-long soil-bag experiment was too short for changes in mass loss to be detected, especially in the B horizon with a low SOM content. On the other hand, C in the bulk soil and soil fractions had probably been influenced by the gradient in labile C input for a longer time than C in the soil bags because we selected medium-sized wood ant nests that could have been already inhabited for several years or even decades (Hölldobler and Wilson 1990).

The soil microbial and faunal community compositions did not show a consistent response to distance from the nest, although we expected relative abundances and activity to be higher at 70 m than at 4 m. Contrary to our expectation, C_{mic} content was higher near than away from the nest in the B horizon. Apparently, labile C stocks in soil fractions, which were also higher near the nest in the B horizon, are more important for increasing microbial biomass than labile C inputs. This explanation is not inconsistent with our finding of an increased decomposition rate and reduced SOC stock distant from the nest because decomposition can be increased via the priming effect without changing the C_{mic} content (Blagodatskaya and Kuzyakov 2013). The effect of distance was also apparent in the abundances of actinobacteria, G^+ bacteria, Oribatida, and Prostigmata. The ability of these microbial and faunal groups to decompose recalcitrant SOM (Gerson 1969; Seifert et al. 2011; Stefaniak and Seniczak 1983) may help explain why they were more abundant closer to the nest where the labile C input was lower. Similar results were found by Maraun et al. (2001), who reported that the abundance of oribatid mites was decreased by glucose-addition treatments. Moreover, the feces of soil fauna contribute to the production of protected microaggregates (Wolters 2000) with reduced decomposability (Six et al. 2000, 2002), which could explain the lack of changes in microaggregate-associated C fractions with the distance from the nest.

Compared to distance from the nest, soil horizon had much larger effects on the abundance of fauna and microorganisms, which was expected because of the substantial differences in pH and SOM content among soil horizons (Jílková et al. 2020). Fauna is generally more abundant in the O horizon than in the A horizon because the O horizon has a higher SOM content (Berg et al. 1998; Ellers et al. 2018; Potapov et al. 2017; Setälä and Aarnio 2002). In addition, fungi are generally tolerant of low pH (Brady and Weil 2002) and are better able to decompose recalcitrant coniferous litter than bacteria (Koranda et al. 2014), and the O horizon was more acidic and contained a higher content of litter mass than the A and B horizons (4.00 ± 0.04, 4.12 ± 0.04, and 4.85 ± 0.04 for pH and 0.56 ± 0.03, 0.13 ± 0.01, and 0.04 ± 0.01 g g^{-1} for OM content, respectively; Jílková et al. 2020). Reduced competition from fungi could explain why bacterial groups were more abundant in the A and B horizons than in the O horizon.

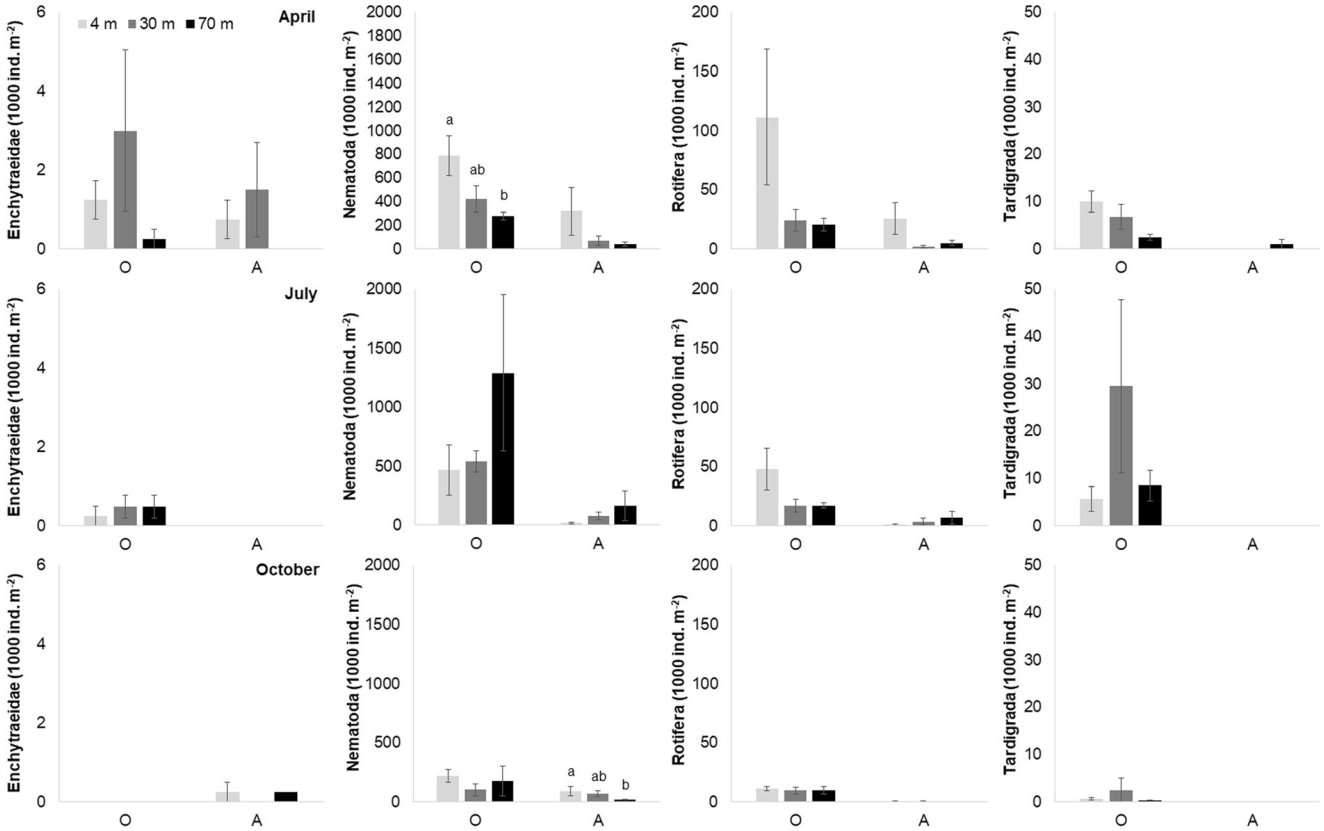

Fig. 4 Numbers of individuals (1000 ind m^{-2}) of microfauna as affected by the distance from the nest (4, 30, and 70 m), soil horizon (O and A), and season (April, July, and October). Values are means ± SE. For each season and horizon, means with different letters are significantly different ($P < 0.05$)

Conclusions

The gradient of labile C input around wood ant nests in a temperate coniferous forest soil affected the rate of litter decomposition and the labile C content in soil fractions, but only in the B horizon. We infer that, because the O and A horizons are less C-limited than the B horizon, the changes in the labile C input along the gradient affected the B horizon more than the surface O and A horizons. On the other hand, community composition and activities of soil organisms were not significantly affected by the gradient in labile C input. Apparently, C stocks in soil fractions are more important for microbial and faunal communities than labile C inputs. Our results show the effects of future changes in labile C inputs to coniferous forest soils because the gradient exploited in our study corresponds to the expected increase in photosynthesis by the end of this century (Norby et al. 1999). Moreover, the labile C input represents an ecologically relevant effect of mixture of compounds (i.e., sugars, amino acids, and phenolics). Although the SOC stock changes very slowly as shown in our study, the natural input of labile C into coniferous forest soils leads to decreases in the SOC stock in the B horizon. By increasing the labile C input to temperate forest soils, future increases in atmospheric CO_2 concentration may therefore lead to a significant loss of SOC in deep soil layers.

Table 3 Results of mixed-effect linear models for mesofauna and microfauna as affected by the distance from the nest (4, 30, and 70 m), soil horizon (O and A), and season (April, July, and October). *F* and *P* values for are shown. *, **, and *** indicate significance at $P < 0.05$, < 0.01, and < 0.001, respectively; NS indicates $P > 0.05$. Three-way interactions were never significant and were not included in the models

Source of variance	df	Oribatida	Prostigmata	Gamasida	Collembola	Enchytraeidae	Nematoda	Rotifera	Tardigrada
Season	2, 49	5.6 **	11.9 ***	0.6 NS	1.7 NS	7.1 **	4.6 *	9.2 ***	20.8 ***
Horizon	2, 49	59.2 ***	30.6 ***	32.9 ***	37.1 ***	3.6 NS	63.5 ***	58.5 ***	226.8 ***
Distance	2, 49	3.4 *	2.7 NS	1.1 NS	0.8 NS	1.1 NS	1.1 NS	0.9 NS	0.3 NS
Season × horizon	2, 49	3.6 *	6.0 **	0.6 NS	0.6 NS	3.6 *	5.0 *	3.8 *	16.9 ***
Season × distance	4, 49	0.6 NS	2.8 *	2.2 NS	2.2 NS	2.5 NS	4.0 **	1.4 NS	1.1 NS
Horizon × distance	4, 49	2.7 NS	1.5 NS	1.4 NS	0.02 NS	0.3 NS	0.3 NS	0.03 NS	2.1 NS

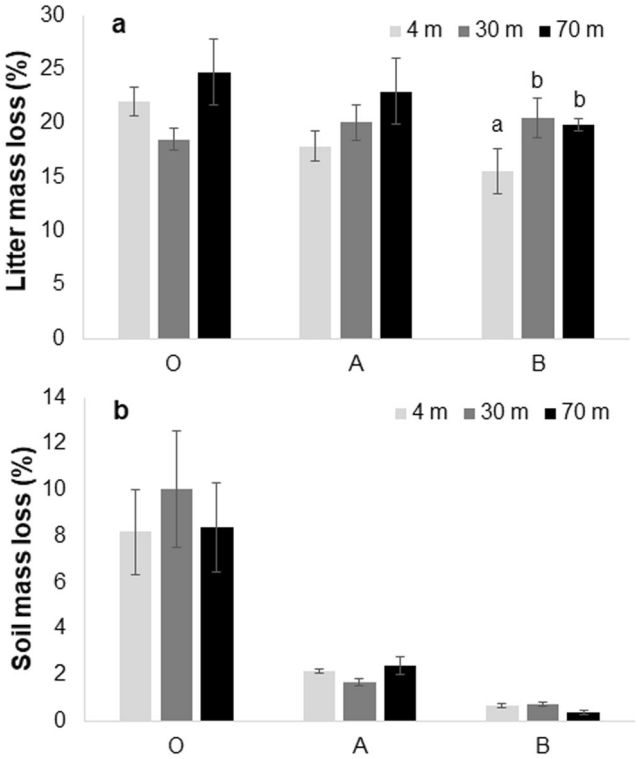

Fig. 5 Litter mass loss (a) and soil mass loss (b) as affected by the distance from the nest (4, 30, or 70 m) and soil horizon (O, A, or B). Mass loss was measured in litter bags and soil bags. Values are means ± SE. For each horizon, means with different letters are significantly different ($P < 0.05$)

Acknowledgments The authors thank Jiří Petrásek, Kristýna Hošková, Ota Rauch, Pavlína Stuchlá, and Jan Hanzelka for help with field sampling and laboratory analyses, and Bruce Jaffee for English revision of the manuscript.

Funding information This study was supported by the Czech Science Foundation (17-08717S), the Czech Academy of Sciences (L200961602), and the Ministry of Education, Youth and Sports of the Czech Republic - MEYS (projects LM2015075, EF16_013/0001782). Some of the equipment used for this study was purchased from the Operational Programme Prague - Competitiveness (Project CZ.2.16/3.1.00/21516). Institutional funding for K. J. was provided by the Center for Geosphere Dynamics (UNCE/SCI/006).

References

Albers D, Schaefer M, Scheu S (2006) Incorporation of plant carbon into the soil animal food web of an arable system. Ecology 87:235–245

Bailey VL, Peacock AD, Smith JL, Bolton H (2002) Relationships between soil microbial biomass determined by chloroform fumigation–extraction, substrate-induced respiration, and phospholipid fatty acid analysis. Soil Biol Biochem 34:1385–1389

Baldock JA, Skjemstad JO (2000) Role of the matrix and minerals in protecting natural organic materials against biological attack. Org Geochem 31:697–710

Berg MP, Kniese JP, Bedaux JJM, Verhoef HA (1998) Dynamics and stratification of functional groups of micro- and mesoarthropods in the organic layer of a scots pine forest. Biol Fertil Soils 26:268–284

Bertin C, Yang XH, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83

Biernath C, Fisher H, Kuzyakov Y (2008) Root uptake of N-containing and N-free low molecular weight organic substances by maize: a $^{14}C/^{15}N$ tracer study. Soil Biol Biochem 40:2237–2245

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biol Fertil Soils 45:115–131

Blagodatskaya E, Kuzyakov Y (2013) Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem 67:192–211

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bradford MA, Fierer N, Reynolds JF (2008) Soil carbon stocks in experimental mesocosms are dependent on the rate of labile carbon, nitrogen and phosphorus inputs to soils. Funct Ecol 22:964–974

Brady NC, Weil RR (2002) The nature and properties of soils. Upper Saddle River, New Jersey, Prentice-Hall

Butler JL, Williams MA, Bottomley PJ, Myrold DD (2003) Microbial community dynamics associated with rhizosphere carbon flow. Appl Environ Microbiol 69:6793–6800

Ceulemans R, Janssens IA, Jach ME (1999) Effects of CO_2 enrichment on trees and forests: lessons to be learned in view of future ecosystem studies. Ann Bot-London 84:577–590

Chigineva NI, Aleksandrova AV, Tiunov AV (2009) The addition of labile carbon alters litter fungal communities and decreases litter decomposition rates. Appl Soil Ecol 42:264–270

Crow SE, Lajtha K, Bowden RD, Yano Y, Brant JB, Caldwell BA, Sulzman EW (2009) Increased coniferous needle inputs accelerate decomposition of soil carbon in an old-growth forest. For Ecol Manag 258:2224–2232

Devetter M (2010) A method for efficient extraction of rotifers (Rotifera) from soils. Pedobiologia 53:115–118

Dixon RK, Brown S, Houghton RA, Solomon AM, Trexler MC, Wisniewski J (1994) Carbon pools and flux of global forest ecosystems. Science 263:185–190

Ellers J, Berg MP, Dias ATC, Fontana S, Ooms A, Moretti M (2018) Diversity in form and function: vertical distribution of soil fauna mediates multidimensional trait variation. J Anim Ecol 87:933–944

Fisher H, Ingwersen J, Kuzyakov Y (2010) Microbial uptake of low-molecular-weight organic substances out-competes sorption in soil. Eur J Soil Sci 61:504–513

Frouz J, Nováková A (2002) The potential effect of high atmospheric CO_2 on soil fungi-invertebrate interactions. Glob Chang Biol 8:339–344

Ganjegunte GK, Condron LM, Clinton PW, Davis MR, Mahieu N (2006) Effects of the addition of forest floor extracts on soil carbon dioxide efflux. Biol Fertil Soils 43:199–207

Gerson U (1969) Moss-arthropod association. Bryologist 72:495–500

Gunina A, Kuzyakov Y (2015) Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. Soil Biol Biochem 90:87–100

Hanson JR, Macalady JL, Harris D, Scow KM (1999) Linking toluene degradation with specific microbial populations in soil. Appl Environ Microbiol 65:5403–5408

Haohao W, Xingkai X, Cuntao D, TuanSheng L, Weiguo C (2017) Effect of carbon and nitrogen addition on nitrous oxide and carbon dioxide fluxes from thawing forest soils. Int Agrophys 31:339–349

Hölldobler B, Wilson EO (1990) The ants. Springer-Verlag, Berlin

Hoosbeek MR, Lukac M, van Dam D, Godbold DL, Velthorst EJ, Biondi FA, Peressotti A, Cotrufo MF, de Angelis P, Scarascia-Mugnozza G (2004) More new carbon in the mineral soil of a poplar plantation

under free air carbon enrichment (POPFACE): cause of increased priming effect? Global Biogeochem Cycles 18:GB1040

Hopkins FM, Filley TR, Gleixner G, Lange M, Top SM, Trumbore SE (2014) Increased belowground carbon inputs and warming promote loss of soil organic carbon through complementary microbial responses. Soil Biol Biochem 76:57–69

Hyvönen R, Ågren GI, Linder S, Persson T, Cotrufo MF, Ekblad A, Freeman M, Grelle A, Janssens IA, Jarvis PG, Kellomäki S, Lindroth A, Loustau D, Lundmark T, Norby RJ, Oren R, Pilegaard K, Ryan MG, Sigurdsson BD, Strömgren M, van Oijen M, Wallin G (2007) The likely impact of elevated [CO_2], nitrogen deposition, increased temperature and management on carbon sequestration in temperate and boreal forest ecosystems: a literature review. New Phytol 173:463–480

Isidorov VA, Smolewska M, Purzynska-Pugacewicz A, Tyszkiewicz Z (2010) Chemical composition of volatile and extractive compounds of pine and spruce leaf litter in the initial stages of decomposition. Biogeosciences 7:2785–2794

Jílková V, Cajthaml T, Frouz J (2015) Respiration in wood ant (*Formica aquilonia*) nests as affected by altitudinal and seasonal changes in temperature. Soil Biol Biochem 86:50–57

Jílková V, Jandová K, Vacířová A, Kukla J (2020) Gradients of labile carbon inputs into the soil surrounding wood ant nests in a temperate forest. Biol Fertil Soils 56:69–79

Jobbágy EG, Jackson RB (2000) The vertical distribution of soil organic carbon and its relation to climate and vegetation. Ecol Appl 10:423–436

Kalbitz K, Kaiser K, Bargholz J, Dardenne P (2006) Lignin degradation controls the production of dissolved organic matter in decomposing foliar litter. Eur J Soil Sci 57:504–516

Klamer M, Bååth E (2004) Estimation of conversion factors for fungal biomass determination in compost using ergosterol and PLFA 18: 2ω6,9. Soil Biol Biochem 36:57–65

Koranda M, Kaiser C, Fuchslueger L, Kitzler B, Sessitsch A, Zechmeister-Boltenstern S, Richter A (2014) Fungal and bacterial utilization of organic substrates depends on substrate complexity and N availability. FEMS Microbiol Ecol 87:142–152

Kuzyakov Y (2010) Priming effects: interactions between living and dead organic matter. Soil Biol Biochem 42:1363–1371

Kuzyakov Y, Friedel JK, Stahr K (2000) Review of mechanisms and quantification of priming effects. Soil Biol Biochem 32:1485–1498

Maraun M, Alphei J, Beste P, Bonkowski M, Buryn R, Migge S, Peter M, Schaefer M, Scheu S (2001) Indirect effects of carbon and nutrient amendments on the soil meso- and microfauna of a beechwood. Biol Fertil Soils 34:222–229

Marshall VG (1972) Comparison of two methods of estimating efficiency of funel extractors for soil microarthropods. Soil Biol Biochem 4: 417–426

Medlyn BE, Berbigier P, Clement R, Grelle A, Loustau D, Linder S, Wingate L, Jarvis PG, Sigurdsson BD, McMurtrie RE (2005) The carbon balance of coniferous forests growing in contrasting climatic conditions: a model-based analysis. Agric For Meteorol 131:97–124

Melillo JM, Butler S, Johnson J, Mohan J, Steudler P, Lux H, Burrows E, Bowles F, Smith R, Scott L, Vario C, Hill T, Burton A, Zhou Y-M, Tang J (2011) Soil warming, carbon–nitrogen interactions, and forest carbon budgets. PNAS 108:9508–9512

Miles P (2000) Vzácní mravenci v CHKO Blanský les. Formica 3:34–41

Nieminen JK, Pohjola P (2014) Labile carbon addition affects soil organisms and N availability but not cellulose decomposition in clear-cut Norway spruce forests. Boreal Environ Res 19:257–266

Norby RJ, Wullschleger SD, Gunderson CA, Johnson DW, Ceulemans R (1999) Tree responses to rising CO_2 in field experiments: implications for the future forest. Plant Cell Environ 22:683–714

Page AL (1982) Methods of soil analysis. Part 2. Chemical and microbiological properties. American Society of Agronomy

Potapov AM, Goncharov AA, Semenina EE, Korotkevich AY, Tsurikov SM, Rozanova OL, Anichkin AE, Zuev AG, Samoylova ES, Semenyuk II, Yevdokimov IV, Tiunov AV (2017) Arthropods in the subsoil: abundance and vertical distribution as related to soil organic matter, microbial biomass and plant roots. Eur J Soil Biol 82:88–97

Qiao N, Schaefer D, Blagodatskaya E, Zou X, Xu X, Kuzyakov Y (2014) Labile carbon retention compensates for CO_2 released by priming in forest soils. Glob Chang Biol 20:1943–1954

Ruf A, Kuzyakov Y, Lopatovskaya O (2006) Carbon fluxes in soil food webs of increasing complexity revealed by ^{14}C labelling and ^{13}C natural abundance. Soil Biol Biochem 38:2390–2400

Scheu S (2001) Plants and generalist predators as links between the below-ground and above-ground system. Basic Appl Ecol 2:3–13

Schlesinger WH (1997) Biogeochemistry, an analysis of global climate change. Academic Press, San Diego, CA, USA/London, UK

Seifert A-G, Trumbore S, Xu X, Zhang D, Kothe E, Gleixner G (2011) Variable effects of labile carbon on the carbon use of different microbial groups in black slate degradation. Geochim Cosmochim Acta 75:2557–2570

Setälä H, Aarnio T (2002) Vertical stratification and trophic interactions among organisms of a soil decomposer food web - a field experiment using ^{15}N as a tool. Eur J Soil Biol 38:29–34

Six J, Elliott ET, Paustian K, Doran JW (1998) Aggregation and soil organic matter accumulation in cultivated and native grassland soils. Soil Sci Soc Am J 62:1367–1377

Six J, Elliott ET, Paustian K (2000) Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. Soil Biol Biochem 32:2099–2103

Six J, Conant RT, Paul EA, Paustian K (2002) Stabilization mechanisms of soil organic matter: implications for C-saturation of soils. Plant Soil 241:155–176

Stefaniak O, Seniczak S (1983) Intestinal microflora of different feeding groups of soil mites (Acarida, Oribatida). In: Lebrun PH, André HM, De Medts A, Gregoire-Wibo C, Wauthy G (Eds) New trends in soil biology. Louvain, Belgium, pp 622–624

Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO_2 efflux in an old growth coniferous forest. Biogeochemistry 73:231–256

Wang Q, Wang Y, Wang S, He T, Liu L (2014) Fresh carbon and nitrogen inputs alter organic carbon mineralization and microbial community in forest deep soil layers. Soil Biol Biochem 72:145–151

Wang H, Xu W, Hu G, Dai W, Jiang P, Bai E (2015) The priming effect of soluble carbon inputs in organic and mineral soils from a temperate forest. Oecologia 178:1239–1250

Wiesmeier M, Prietzel J, Barthold F, Spörlein P, Geuß U, Hangen E, Reischl A, Schilling B, von Lützow M, Kögel-Knabner I (2013) Storage and drivers of organic carbon in forest soils of southeast Germany (Bavaria) – implications for carbon sequestration. For Ecol Manag 295:162–172

Wolters V (2000) Invertebrate control of soil organic matter stability. Biol Fertil Soils 31:1–19

Zelles L (1999) Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. Biol Fertil Soils 29:111–129