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REGULAR ARTICLE

Contribution of litter layer to soil greenhouse gas emissions in a temperate beech forest

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Abstract

Background and aims The litter layer is a major source of CO_2, and it also influences soil-atmosphere exchange of N_2O and CH_4. So far, it is not clear how much of soil greenhouse gas (GHG) emission derives from the litter layer itself or is litter-induced. The present study investigates how the litter layer controls soil GHG fluxes and microbial decomposer communities in a temperate beech forest.

Methods We removed the litter layer in an Austrian beech forest and studied responses of soil CO_2, CH_4 and N_2O fluxes and the microbial community via phospholipid fatty acids (PLFA). Soil GHG fluxes were determined with static chambers on 22 occasions from July 2012 to February 2013, and soil samples collected at 8 sampling events.

Results Litter removal reduced CO_2 emissions by 30 % and increased temperature sensitivity (Q_{10}) of CO_2 fluxes. Diffusion of CH_4 into soil was facilitated by litter removal and CH_4 uptake increased by 16 %. This effect was strongest in autumn and winter when soil moisture was high. Soils without litter turned from net N_2O sources to slight N_2O sinks because N_2O emissions peaked after rain events in summer and autumn, which was not the case in litter-removal plots. Microbial composition was only transiently affected by litter removal but strongly influenced by seasonality.

Conclusions Litter layers must be considered in calculating forest GHG budgets, and their influence on temperature sensitivity of soil GHG fluxes taken into account for future climate scenarios.

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Keywords Litter removal · Seasonality · CO_2 · CH_4 · N_2O · PLFA

Abbreviations

LR	Litter removal
CO_2	Carbon dioxide
CH_4	Methane
N_2O	Nitrous oxide
GHG	Greenhouse gases
SOC	Soil organic carbon
TN	Total nitrogen
C_{mic}	Microbial carbon
N_{mic}	Microbial nitrogen
NH_4^+	Ammonium

NO_3^-	Nitrate
PO_4^{3-}	Phosphate
WSS	Water-soluble sugars
VWC	Soil volumetric water content
T_{soil}	Soil temperature
DaLR	Day after litter removal
PLFA	Phospholipid fatty acids
gram-	Gram-negative bacteria
gram+	Gram-positive bacteria
Q_{10}	Temperature sensitivity
CCA	Canonical correspondence analysis

Introduction

Forest soils play an important role in controlling global greenhouse gas (GHG) budgets because they act mostly as carbon dioxide (CO_2) sources, methane (CH_4) sinks and nitrous oxide (N_2O) sources (IPCC 2013). Soil microbial communities strongly influence soil GHG fluxes (Conrad 1996; Schimel and Gulledge 1998), and are typically adapted to the type of plant litter in a certain environment (Ayres et al. 2009; Madritch and Lindroth 2011). Although plant litter contributes the largest input of C and nutrients to forest soils (FAO 2010), there is a lack of knowledge on the explicit impact of the litter layer on forest soil GHG fluxes. Atmospheric CO_2 is the major driver of global warming, and CH_4 and N_2O are potent GHGs with 100-year global warming potentials of 28 and 265, respectively (IPCC 2013). Partitioning the contribution of litter and mineral soil to total soil GHG fluxes as well as improving our understanding on how the litter layer influences soil processes and microbial communities will help to reduce uncertainties in biogeochemical models and improve our forecasts of future GHG budgets for terrestrial ecosystems. Because ecosystem GHG sinks can be used to a limited extend to compensate for emission reductions stipulated in the Kyoto protocol (IPCC 2014), a precise quantification of ecosystem C and N budgets is of utmost importance for climate change mitigation.

Forests cover 31 % of land area and contain 652 GtC, 45 % in soils and 11 % in dead wood and litter (FAO 2010). Respiration from plant litter decomposition contributes between 5 and 45 % to total soil CO_2 emissions in temperate forests (Borken and Beese 2005; Bowden et al. 1993; Vose and Bolstad 2007). The litter-inhabiting microbial community in beech forests is dominated by fungi that can decompose litter cellulose and lignin (Schneider et al. 2012). Removing the litter might decrease the fungi:bacteria (F:B) ratio in the soil. Because most fungi have a higher C use efficiency (CUE) than bacteria (Keiblinger et al. 2010), a shift in the F:B ratio is likely to affect soil CO_2 emissions.

Furthermore, temperate forests are considered to be important CH_4 sinks through the consumption of CH_4 by methanotrophic bacteria in well-aerated forest soils (Dalal and Allen 2008; Le Mer and Roger 2001). Litter itself does apparently not produce or consume CH_4 (Dong et al. 1998; Reith et al. 2002; Smith et al. 2000). However, the litter layer has been reported to influence soil CH_4 uptake by controlling gas diffusion into the soil (Peichl et al. 2010; Wang et al. 2013), which can be particularly important in broad-leaved forests like beech (Brumme and Borken 1999). Furthermore, soils that receive high N loads due to N fertilization or atmospheric N deposition often consume less CH_4 than undisturbed soils (Butterbach-Bahl et al. 1998; Macdonald et al. 1997; Steudler et al. 1989) because NH_4^+ inhibits oxidation of CH_4 to CO_2 by methanotrophic bacteria (Bodelier and Laanbroek 2004). However, whether litter N content influences soil CH_4 fluxes, for example via leaching of N to the mineral soil, remains to be demonstrated.

Soils under natural vegetation are mostly regarded as N_2O sources and account for 6.6 Tg N_2O-N yr^{-1} to the global terrestrial N_2O input to the atmosphere (IPCC 2013). How the litter layer affects soil N_2O flux is not clear. Dong et al. (1998) reported that removal of leaf litter/humus layer significantly decreased N_2O emissions in a German deciduous forest, which they attributed primarily to emissions of the humus layer itself. Wieder et al. (2011) found a priming effect of labile C leaching from plant litter on soil N_2O emissions for tropical ecosystems, which can either be a direct result of stimulation of heterotrophic denitrifiers or occur indirectly by increased heterotrophic O_2 consumption and formation of anaerobic microsites in the soil. However, contribution of litter itself to total soil N_2O emissions in temperate forests as well as the importance of dissolved organic carbon (DOC) leaching from litter to mineral soils is not well studied.

The purpose of the present study was to quantify how much of forest soil GHG flux is litter-induced, as well as to investigate how removal of the aboveground litter layer (henceforth referred to as 'litter removal') influences the soil processes and microbial community

composition in the short term. We hypothesized that litter removal (i) reduces soil concentrations of mobile C, N and P, (ii) reduces soil CO_2 efflux, (iii) enhances soil CH_4 uptake, (iv) reduces soil N_2O efflux, and (v) reduces the proportion of fungi in the soil microbial community.

Materials and methods

Study site

The study was conducted in a pure mature beech forest (*Fagus sylvatica* L.) at the 'Rosalia Lehrforst' site, which is part of the 'long-term ecological research' network (LTER-Austria) and is located in the Rosalien Mountains, Austria (47° 42′ 26″ N /16° 17′ 59″ E). The soil at the study site was a pseudo-gleyic Cambisol over metamorphic crystalline bedrock. Mean annual temperature and mean annual precipitation were 6.5 °C and 796 mm, respectively. The study site was at an elevation of 600 m asl and exposed to the west.

Experimental design

Twelve pairs of experimental plots were randomly positioned along a 20 m horizontal line, each consisting of one control and one litter-removal (LR) plot. The litter layer was removed carefully by hand in an area of 0.5 m × 0.5 m from the LR plots in June 2012. Total removed litter accounted for 1.39 kg dw m^{-2}, which contained 0.55 kg C m^{-2}. The bare mineral soil was covered with a black water-permeable textile mat to prevent excessive soil-drying due to litter removal, which allowed us to focus on the influence of nutrient leaching from the litter rather than changes in soil microclimate. A metal mesh cage (25 cm height) was placed over the LR plots to prevent new litter input. On all 24 plots, PVC collars of 20 cm diameter and 10 cm height were inserted carefully 2–3 cm into the ground to be used as closed headspace chambers to collect air samples. Between July 2012 and February 2013 air samples were collected 22 times and soil samples 8 times. Microbial community composition was determined via phospholipid fatty acid (PLFA) analysis at 5 time points.

Soil analysis

Soil samples were collected from all 24 plots with metal cylinders of 4 cm diameter and 5 cm height. At each sampling, 5 soil cores from each plot were taken and pooled together. Before soil cores were taken from control plots, the litter layer was carefully moved aside locally and only mineral soil was sampled to make soil samples from control plots comparable to those from LR plots. At the same time, soil temperature in 5 cm depth was determined with a penetration thermometer (Voltcraft DET3R, Switzerland), and volumetric water content (VWC) was measured with a TDR probe (SM300, Delta-T, UK). Soil samples were transported to the laboratory in Vienna, sieved (<2 mm) and stored at 4 °C for nutrient and microbial biomass analysis, and at −18 °C for PLFA analysis. All soil samples were analyzed for pH, NO_3^-, NH_4^+, PO_4^{3-}, water-soluble sugars (WSS), microbial biomass, and soil organic C (SOC) and total nitrogen (TN) contents. Soil pH was determined with a calibrated pH-meter (WTW 537, Germany) in a suspension of 2 g fresh soil in 25 ml 0.01 *M* $CaCl_2$ (Schinner et al. 1996). Nitrate, NH_4^+ and PO_4^{3-} concentrations were measured in suspensions of 5 g fresh soil in 50 ml 1 *M* KCl with a photometer (Perkin Elmer 2300 EnSpire, USA) as described elsewhere (Hood-Nowotny et al. 2010; Schinner et al. 1996). Hot-water soluble reducing sugars (WSS) were detected with the Prussian-blue method (Schinner and Von Mersi 1990; Slaughter et al. 2001). Microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}) of the samples was calculated as difference of DOC and total dissolved nitrogen (TDN), respectively, before and after chloroform fumigation (Schinner et al. 1996). Soil organic C and TN were quantified on oven-dried (105 °C) soil with an elemental analyzer (NA-1500 Carlo Erba, Italy). Additionally, the textile mat was tested for leaching of C, N and P, and no leaching was detected.

Soil greenhouse gas fluxes

To collect gas samples, the 24 dark chambers (total volume 2.51 L) were closed with air-tight lids and gas samples were collected with a syringe through a rubber septum in the lid 0, 10, 20 and 60 min after chamber closure. 30 ml gas samples were injected into 20 ml pre-evacuated glass vials (clear flat-bottom headspace vials with aluminum crimp caps and grey butyl septa, all from Agilent Technologies, Austria) and transported to the

lab. Gas samples were stored at air temperature and analyzed within 1 week. Concentrations of CO_2, CH_4 and N_2O of all gas samples were determined with an Agilent GC-system (Agilent Technologies). Detector 1 was an electron capture detector (ECD) for N_2O measurements, and detector 2 was a flame ionization detector (FID) with Ni-methanizer to quantify CO_2 and CH_4 (all Agilent Technologies, Austria). For calibration, gas mixes of CO_2, CH_4 and N_2O in N_2-gas in 3 different concentrations (CO_2 250, 500, 1000 ppm; CH_4 1, 2, 4 ppm; N_2O 0.5, 2.5, 5 ppm, respectively) were used (Linde Gas, Austria). Limit of detection (LoD) of the chamber measurements was 3.6 mg CO_2-C m^{-2} h^{-1}, 9.2 μg CH_4-C m^{-2} h^{-1} and 10.1 μg N_2O-N m^{-2} h^{-1}, respectively (Parkin et al. 2012). Because N_2O fluxes from temperate forest soils are known to be highly variable in time and space, with high fluxes during "hot moments" such as drying-rewetting or freeze-thaw events, and low fluxes during the rest of the year (Groffman et al. 2009). Therefore, in the present study fluxes below the LoD were not excluded from the calculation of average fluxes over the study period, because this would have caused a bias towards higher emissions. Nevertheless, the reader should be aware that values below LoD bear a high analytical uncertainty.

Hourly GHG flux rates for each chamber were calculated based on Eq. (1) as described by Metcalfe et al. (2007),

$$\text{GHG flux} = \Delta C/\Delta t * 273.15/(T_{air} + 273.15) * p/1000 * M/22.41 * V/A \quad (1)$$

Where *GHG flux* is the flux of the respective greenhouse gas, $\Delta C/\Delta t$ is the concentration change (ppm for CO_2, ppb for CH_4 and N_2O) over time (h), T_{air} is air temperature (°C), p is atmospheric pressure (Pa), M is molecular weight (g), 22.41 is the molar volume of an ideal gas at Standard Temperature and Pressure (1 mol^{-1}), V is the chamber volume (m^3) and A the chamber area (m^2). The term (T_{air} + 273.15) is used to convert air temperature from degree Celsius to Kelvin. For calculation of CO_2 and CH_4 fluxes, M is 12.01 g (the molecular weight of C) and units are mg CO_2-C h^{-1} m^{-2} and μg CH_4-C h^{-1} m^{-2}, respectively. For calculation of N_2O flux, M is 28.02 g (the molecular weight of 2 N atoms) and units are μg N_2O-N h^{-1} m^{-2}. Concentration changes over time were determined with quadratic best-fit equations for CO_2 and N_2O, and an exponential best-fit equation for CH_4. Greenhouse gas fluxes were discharged if regression coefficients (r^2) were below 0.70 for CH_4 and N_2O, and below 0.90 for CO_2 (Barton et al. 2008; Chadwick et al. 2014; Unteregelsbacher et al. 2013). Positive fluxes represent net GHG emissions, negative fluxes represent net GHG uptake. Greenhouse gas fluxes of control and LR plots were averaged for each sampling event and are given together with standard errors (n = 12 per treatment). Litter-induced GHG flux was calculated as the difference between GHG flux from control plots (soil & litter) and LR plots (soil only):

$$\text{Litter-induced GHG flux} = \text{GHG flux}_{control} - \text{GHG flux}_{LR} \quad (2)$$

Temperature sensitivity values (Q_{10}) were calculated for soil GHG fluxes that were significantly correlated with soil temperature after a Lloyd & Taylor function (Eq. 3) according to Tuomi et al. (2008):

$$\text{GHG flux} = a * \exp((E/(283.15*8.314))*(1-283.15/(T_{soil} + 273.15))) \quad (3)$$

with a and E as fitted parameters, T_{soil} the soil temperature (°C), which is converted to Kelvin by adding 273.15, 8.314 is the universal gas constant (J mol^{-1} K^{-1}), and 283.15 is some reference temperature (10 °C, see also Lloyd and Taylor 1994).

Phospholipid fatty acid (PLFA) analysis

Phospholipid fatty acids were analyzed in pooled soil samples for reasons of feasibility (from the 12 soil samples per treatment, 4 were combined to one composite sample, which resulted in 3 composite samples per treatment and time point). Phospholipid fatty acids were extracted after an adapted protocol of the Bligh and Dyer method (Frostegård et al. 1991) as described elsewhere (Brandstätter et al. 2013; Djukic et al. 2010). Briefly, 2 g field-moist soil were extracted overnight in the dark with chloroform:methanol:citrate buffer (1:2:0.8) and chloroform:methanol (1:2), fractionated by sequential elution with chloroform, acetone and methanol on silica solid-phase columns (Isolute SI 500 mg 3 ml^{-1}, Biotage, Sweden) to separate phospholipids from neutral lipid fatty acids and glycolipids. Samples were methylated with methanol:toluol (1:1), 0.2 *M* methanolic KOH and 1 *M* acetic acid. Phospholipids where re-dissolved in 200 μl iso-octane and analyzed with an HP 6980 series GC-system and 7683 series injector and auto-sampler on an HP-5 50 m

capillary column (all Hewlett Packard, USA) using a flame ionization (FID) detector. A mix of bacterial acid methyl esters (Supelco BAME CP Mix # 47080-U, Sigma-Aldrich, USA) was used as qualitative standard to identify PLFAs. Concentrations of individual PLFAs were quantified relative to the internal standard nonadecanoate fatty acid (19:0, 20 mg l^{-1}).

Absolute amounts of PLFAs are given in μmol PLFA g^{-1} SOC. The PLFAs i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 and 10Me18:0 were used as markers for gram+ bacteria, cy17:0, cy19:0, 16:1ω5c, 16:1ω7c, 14:0, 15:0, 17:0 for gram- bacteria, 10Me16:0 and 10Me17:0 for unspecific bacteria, and 18:2ω6,9 for fungi (Baath 2003; Djukic et al. 2010, 2013; Zelles 1999). Total bacterial PLFAs were calculated from the sum of gram+, gram- and unspecific bacterial markers. Bacteria:fungi ratio was calculated as the sum of bacterial PLFAs divided by the fungal PLFA 18:2ω6,9.

Statistical analysis

To identify effects of time and litter removal, first a two-way ANOVA was used to check for interactions between factors. If interactions were found, the dataset was split into control and LR subsets, and one-way ANOVA followed by Tukey's *post hoc* test was employed to identify differences between time points. Differences between treatments were analyzed by separate *t*-tests for each time point. Homogeneity of variance was tested with Levene's test, and data were log-transformed if necessary. If transformation did not ensure homogeneity of variance, robust ANOVA as described by Wilcox (2005) was employed. Microbial community composition was analyzed by canonical correspondence analysis (CCA), using the mole percentage of PLFAs as community matrix and soil parameters and time as constraining factors. Interactions between soil GHG fluxes and soil temperature and moisture were analyzed on data from 22 gas samplings by Spearman's rank correlation with Benjamini & Hochberg correction to test for false positives (type I error) in multiple comparisons (Benjamini and Hochberg 1995). Statistical analysis was conducted with Statgraphics (StatPoint Technologies, United States), SigmaPlot (Systat Software, USA), and R 3.0.2 using packages "vegan" for CCA (Oksanen et al. 2014) and "WRS" for robust ANOVA (Wilcox 2005).

Results

Soil properties

Average soil temperatures were 11.2 ± 1.4 °C and 11.1 ± 1.4 °C between July 2012 and February 2013 for control and LR plots, respectively, and were not significantly altered by litter removal (Fig. 1a). Soil temperature changed according to seasons and decreased from 18 °C during July and August to 2–5 °C in December to February. Volumetric soil water content (Fig. 1a), which averaged 22.4 ± 1.8 % and 23.5 ± 2.1 % for control and LR plots, respectively, was also not significantly affected by litter removal and increased from July to February, with a large peak in the first 2 weeks of August 2012 due to strong rainfall events. Soils at our site were strongly acidic with a mean soil pH of 3.9 ± 0.1, which was not affected by litter removal. Bulk density was 0.595 ± 0.143 g m^{-3}.

Soil nutrients were only affected by litter removal at the start of the experiment (Table 1). One week after removing the litter layer, NH_4^+ increased by 134 % from 302 ± 26 mg N m^{-2} in controls to 710 ± 20 mg N m^{-2} in LR plots. Stocks of SOC (2.1 ± 0.24 kg C m^{-2}) and TN (0.11 ± 0.01 kg N m^{-2}) in the uppermost 5 cm were not influenced by litter removal. At the consecutive samplings, concentrations of NO_3^-, NH_4^+, PO_4^{3-} and WSS did not differ between treatments (Table 2).

Soil greenhouse gas fluxes

Litter removal significantly decreased CO_2 fluxes from soil by 29.9 % (Fig. 1b, Table 2). On average, control plots emitted 128 ± 13 mg CO_2-C h^{-1} m^{-2}, whereas LR plots respired 90 ± 10 mg CO_2-C h^{-1} m^{-2}. Soil CO_2 fluxes of both control and LR plots followed the seasonal trend of soil temperature and decreased from July to February. Absolute litter-induced CO_2 fluxes (difference between control and LR plots) decreased from July 2012 to February 2013, and relative contribution of litter-induced to total soil CO_2 efflux ranged from 15.6 to 46.1 %.

The forest soil acted as atmospheric CH_4 sink during the entire study period (Fig. 1c). Litter removal significantly increased soil CH_4 uptake by 16.0 % (i.e., CH_4 fluxes were 16.0 % more negative) with average CH_4 uptakes of 40.0 ± 2.3 μg CH_4-C h^{-1} m^{-2} in control plots and 46.4 ± 2.6 μg CH_4-C h^{-1} m^{-2} in LR plots. Differences between control and LR plots where large

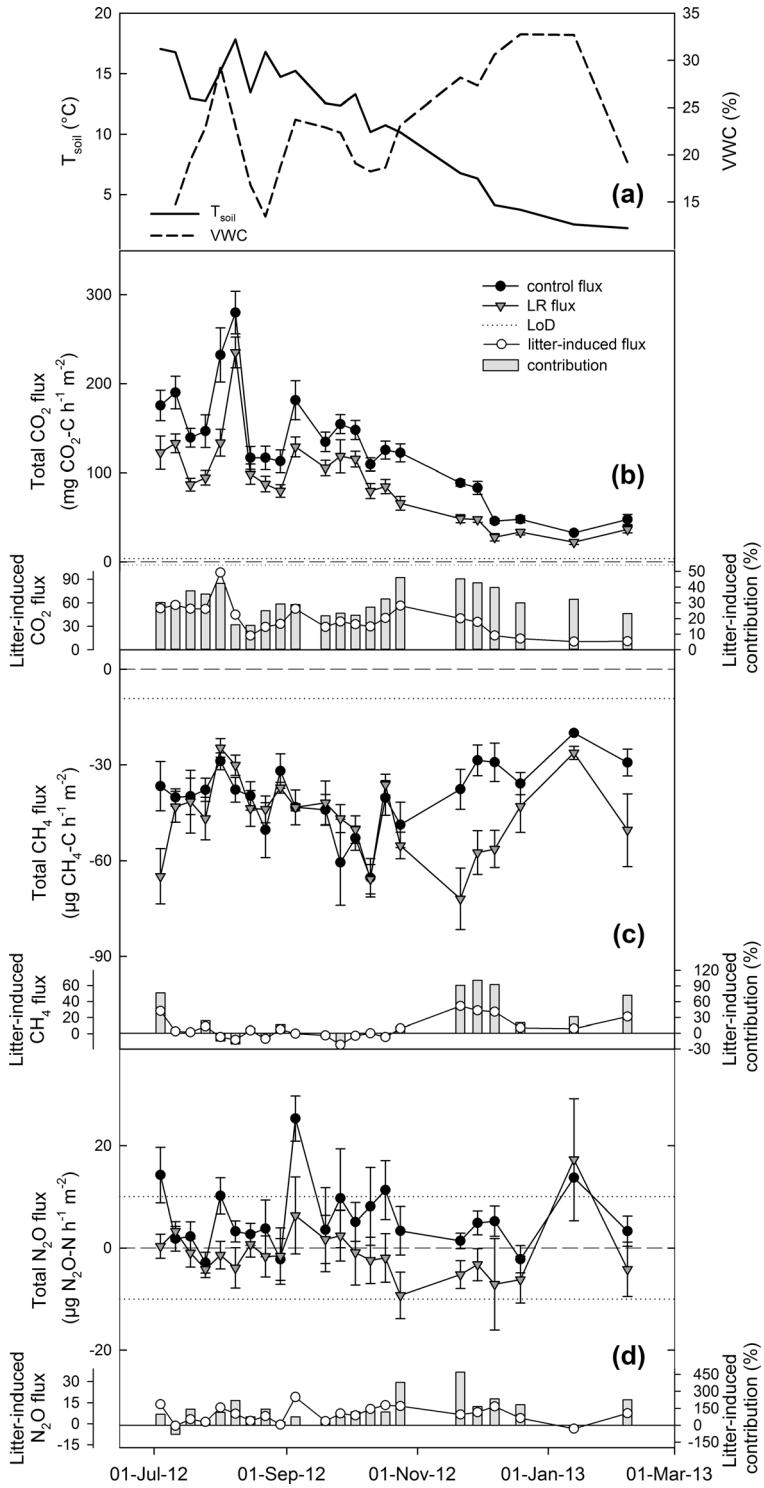

Fig. 1 **a**, soil temperature (T_{soil}, *solid line*) and soil volumetric water content (VWC, *dashed line*) in the experimental plots; **b**–**d**, total greenhouse gas (GHG) flux from control (●) and litter-removal (▽) plots as well as litter-induced GHG flux (○) and contribution of litter-induced to total GHG flux (*grey bars*) (mean ± SE, n = 12): **b**, CO_2; **c**, CH_4; **d**, N_2O. Limit of Detection (LoD, dotted line) of the used GC system was 3.6 mg CO_2-C m^{-2} h^{-1}, 9.2 µg CH_4-C m^{-2} h^{-1} and 10.1 µg N_2O-N m^{-2} h^{-1}, respectively. Positive fluxes (CO_2 and N_2O) indicate soil GHG emissions, negative fluxes (CH_4 and N_2O) indicate soil GHG uptake. Litter-induced flux was calculated as difference between average control and litter-removal GHG fluxes, therefore no standard errors are given

at the beginning of the experiment and in the time from November 2012 to February 2013, with highest absolute litter-induced CH_4 fluxes of 28.2 µg CH_4-C h^{-1} m^{-2} in July 2012 and 34.3 µg CH_4-C h^{-1} m^{-2} in November 2012, which correspond to a 76.8 and 100.9 % increase in CH_4 uptake, respectively, if the litter layer was

Table 1 Soil chemical properties of the uppermost 5 cm from July 2012 until February 2013 in control and litter-removal (LR) plots

Date		C_{mic} (g C m^{-2})		N_{mic} (g N m^{-2})		NO_3^- (mg N m^{-2})		NH_4^+ (mg N m^{-2})		PO_4^{3-} (mg P m^{-2})		WSS (mg Glc-equ m^{-2})	
02-Jul-12	control	**27.0**[a]	±2.3	4.3	±0.4	387	±111	**302**[a]	±25	42.5	±11.2	29.3	±3.7
	LR	**46.6**[b]	±8.2	8.0	±1.8	470	±128	**710**[b]	±20	53.2	±10.8	33.8	±3.9
15-Jul-12	control	28.5	±4.0	8.2	±0.8	124	±40	225	±28	59.6	±12.5	43.0	±3.6
	LR	24.7	±4.7	7.1	±0.8	126	±42	284	±21	55.6	±8.9	36.6	±3.4
30-Jul-12	control	39.8	±6.1	5.8	±1.1	628	±230	449	±49	59.7	±8.2	42.7	±3.9
	LR	30.5	±3.1	4.2	±0.5	284	±97	393	±45	46.8	±3.5	39.8	±3.6
20-Aug-12	control	39.2	±3.7	5.8	±0.7	489	±140	336	±26	44.9	±5.7	19.8	±1.8
	LR	39.6	±5.4	6.2	±1.0	332	±86	402	±42	49.0	±8.4	19.7	±2.0
24-Sep-12	control	43.3	±4.5	4.7	±0.7	427	±210	342	±35	43.1	±4.8	16.0	±1.1
	LR	43.8	±4.9	5.1	±0.7	283	±90	345	±42	40.6	±4.6	15.1	±1.4
15-Oct-12	control	48.6	±6.2	4.8	±1.0	431	±116	365	±52	32.3	±3.7	13.2	±1.2
	LR	56.7	±5.2	6.7	±0.9	467	±107	372	±31	30.8	±5.7	11.2	±1.0
05-Dec-12	control	38.6	±5.0	6.4	±0.8	17.1	±5.0	127	±10	49.7	±6.1	27.1	±3.7
	LR	34.9	±2.9	5.7	±0.5	12.7	±8.1	106	±7	37.6	±3.3	21.8	±2.5
05-Feb-13	control	38.3	±3.2	6.2	±0.6	182	±65	273	±39	35.3	±3.7	22.8	±1.9
	LR	35.9	±3.6	5.9	±0.6	94.5	±27.2	256	±27	28.7	±4.1	19.0	±1.8

C_{mic} and N_{mic} microbial carbon and nitrogen, *WSS* water-soluble sugars (mg Glucose-equivalents m^{-2})

Data are means ± SE with $n = 12$ for each treatment. Bold values indicate significant difference between treatments (*t*-test; $P < 0.05$)

Table 2 Results from two-way ANOVA showing effects of time and litter removal on soil gas fluxes, soil parameters, and microbial groups detected by phospholipid fatty acid (PLFA) analysis. *T_{soil}* soil temperature at 5 cm, *VWC* soil volumetric water content, *C_{mic}* microbial carbon, *N_{mic}* microbial nitrogen, *WSS* water-soluble sugars

	Time		Litter removal		Time x Litter removal	
	F	p	F	p	F	p
CO_2	51.3	***	146	***	0.95	ns
CH_4	4.71	***	12.4	***	2.27	**
N_2O	1.92	*	22.8	***	0.62	ns
T_{soil}	107649	***	0.02	ns	46.2	***
VWC	52.8	***	2.91	ns	0.51	ns
C_{mic}	4.72	***	0.26	ns	1.73	ns
N_{mic}	1.92	ns	0.67	ns	2.00	ns
NO_3^-	108	***	0.01	ns	4.97	ns
NH_4^+	24.7	***	2.45	ns	2.31	*
PO_4^{3-}	21.9	*	0.16	ns	5.20	ns
WSS	2.45	*	0.38	ns	1.41	ns
pH	5.33	***	0.09	ns	2.10	*
Total PLFAs	2.20	ns	0.50	ns	3.49	*
Gram + bacteria	1.36	ns	0.68	ns	3.18	*
Gram- bacteria	2.36	ns	0.15	ns	4.06	*
Fungi	3.28	*	0.32	ns	0.59	ns

Soil gas fluxes and soil parameters, $n = 12$; microbial groups, $n = 3$. *Asterisks* indicate levels of significance (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$)

removed. At the other sampling dates between July and October 2012, soil CH_4 uptake was of similar magnitude in control and LR plots.

Average N_2O fluxes were 117.5 % lower in LR than control plots (Fig. 1d), with control plots acting as N_2O sources (5.72 ± 1.38 μg N_2O-N h^{-1} m^{-2}), while LR led to an uptake of atmospheric N_2O of 1.00 ± 1.16 μg N_2O-N h^{-1} m^{-2}. However, soils under both treatments switched between being N_2O sources and N_2O sinks during the study period. In control plots, we observed three N_2O emission peaks on 01-Aug-2012, 05-Sep-2012 and 17-Oct-2012, where VWC had rapidly increased after periods of dry conditions. Although VWC was similar in LR plots on these dates, N_2O fluxes did not increase. Furthermore, on 13-Jan-2013 high N_2O emissions were detected in both treatments under a thin snow and ice cover (~1 cm). On the other sampling dates, N_2O fluxes were below the LoD (Fig. 1d).

Carbon dioxide fluxes were positively correlated with soil temperature in both treatments (control: $r = 0.86$, $P < 0.01$; LR: $r = 0.84$, $P < 0.01$) and negatively correlated with VWC in LR plots ($r = -0.51$, $P < 0.05$). Methane fluxes were positively related to VWC only in control plots ($r = 0.53$, $P < 0.05$), whereas N_2O fluxes did not reveal any significant correlations with soil temperature or VWC. Soil temperature and VWC were negatively correlated over the study period in both control and LR plots (both $r = -0.64$, $P < 0.01$).

Temperature sensitivities of CO_2 fluxes (Q_{10}, Fig. 2) decreased with increasing soil temperature in both treatments. At 11 °C, which was the mean soil temperature during the study period, the Q_{10} calculated from CO_2 fluxes at 11 and 21 °C was 2.45 ± 0.07 in control plots and 2.86 ± 0.09 in LR plots. Calculated over the observed T_{soil} range (4–18 °C), removing the litter significantly increased Q_{10} values (t-test, $t = -13.7$, $p < 0.001$).

Soil microbial community composition

One week after removing the litter layer, C_{mic} increased by 72.6 % due to litter removal (Table 1). At all following sampling dates, C_{mic} in LR plots was not significantly different from controls. Furthermore, seasonal changes of C_{mic} were observed, with highest values in October, whereas N_{mic} was relatively stable throughout the study period.

Similarly to C_{mic}, the total sum of microbial PLFAs was affected by litter removal at the first sampling date and increased by 37.3 % 1 week after litter removal (Fig. 3). On this date, PLFA markers for gram+ (+36.9 %) and gram- (+30.9 %) bacteria were also significantly increased in LR plots. At the other samplings dates, no significant differences between treatments were found. However, seasonal changes in PLFA groups were detected in control plots, with highest concentrations of bacterial PLFA markers in August and February and lowest concentrations in July. The fungal PLFA marker 18:2ω6,9 constantly increased from July to February.

The influence of environmental parameters on total microbial community variation as expressed

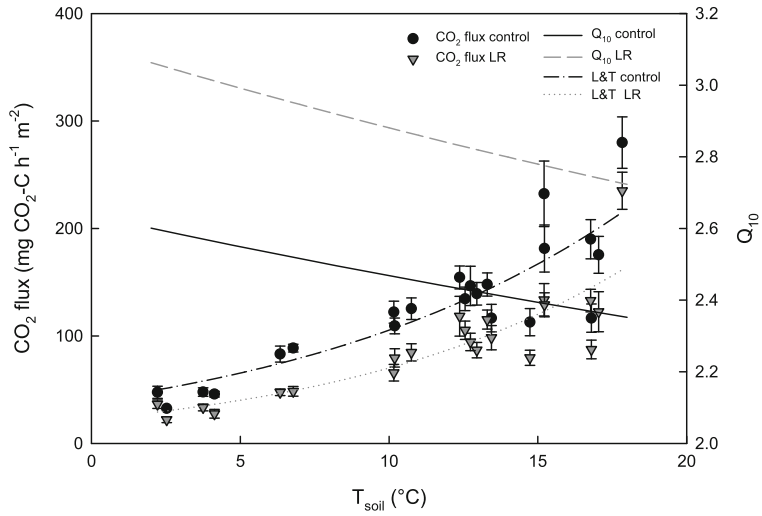

Fig. 2 CO_2 flux (mean ± SE, $n = 12$) in control (●) and litter-removal (▽) plots, and temperature sensitivity (Q_{10}) of CO_2 flux in control (*black solid line*) and litter-removal (*grey dashed line*) plots. Relationship between CO_2 flux and T_{soil} was best described by a Lloyd & Taylor (F&T) function ($r^2 = 0.74$, $P < 0.001$ for control plots, *black dashed-dotted line*; $r^2 = 0.73$, $P < 0.001$ for litter-removal plots, *grey dotted line*)

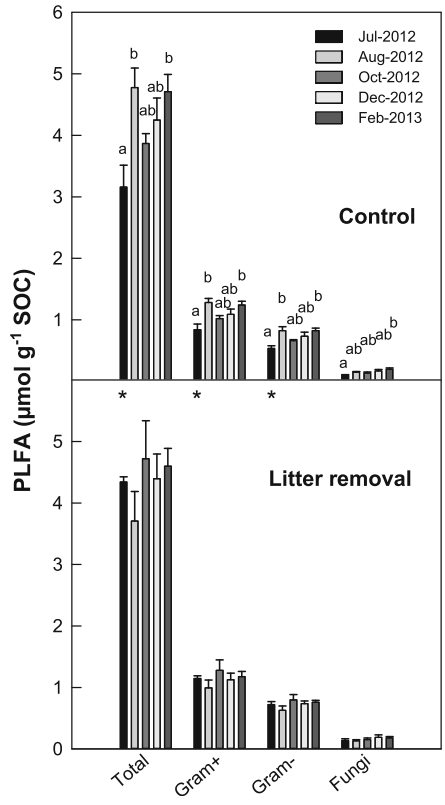

Fig. 3 Concentrations of total, gram + bacterial, gram- bacterial and fungal phospholipid fatty acid (PLFA) markers in soil from control (*upper panel*) and litter-removal (*lower panel*) plots from July 2012 to February 2013. *Asterisks* indicate significant differences between treatments at the respective time points (*t*-test; *, $P < 0.05$), *letters* indicate significant differences between time points for the respective treatment (one-way ANOVA, no time effect for litter removal was found). Given are means ± SE ($n = 3$)

by the constrained variability of the CCA was 67.9 %, split in 41.1 % and 17.6 % for the CCA1 and CCA2, respectively. Both CCA1 and CCA2 were significant ($P < 0.001$, permutation test). The abundance (mol%) of the fungal PLFA 18:2ω6,9 was positively related to VWC and PO_4^{3-} and negatively to C_{mic} and N_{mic}, SOC, TN and NO_3^- (Fig. 4a). The abundance of bacterial PLFAs (gram+, gram- and general bacteria) was positively related to soil temperature, NO_3^-, NH_4^+, SOC and TN, and negatively to pH, VWC and DaLR. PLFA scores (Fig. 4b), an indicator of species composition, showed that differences between treatments were only significant at the first two sampling dates. There was a clear separation between sampling time points showing a shift from July 2012 to February 2013.

Discussion

Soil properties

We hypothesized that litter removal affects concentrations of mobile C and nutrients in the mineral soil (Hypothesis i) because litter is a major source for soil nutrients, and depolymerization of litter compounds yields mobile molecules like sugars, phenols, amino acids and NO_3^- which are water-soluble and prone to leaching into the mineral soil. However, our results did not confirm this assumption. We only found a temporary increase of NH_4^+ at the first sampling date, which presumably was a disturbance effect of the litter removal in the week before. Surprisingly, we found no changes in NO_3^-, NH_4^+, PO_4^{3-} or WSS at any other sampling date. Similar results were reported by Xu et al. (2013), who conducted a meta-analysis on 70 *in situ* litter manipulation experiments across various ecosystems and climatic regions. They discovered that litter removal had no influence on concentrations of DOC, extractable inorganic N (EIN) and extractable P in mineral soils of temperate forests. Litter-derived DOC can be quickly mineralized by soil microbial communities (Kalbitz et al. 2003) and adsorbed to the soil mineral matrix (Guelland et al. 2013). Mobile N forms like NO_3^-, NH_4^+ and amino acids are quickly immobilized by microorganisms and plant roots in the mineral soil (Inselsbacher et al. 2010). Litter-derived P can be adsorbed to the mineral matrix (Tiessen 2008) or taken up by plant roots before it enters the mineral soil (Attiwill and Adams 1993). It is therefore possible that because DOC, inorganic N and P were either adsorbed to the mineral matrix or turned over quickly, in the present study changes in these pools caused by litter manipulation were not detectable with standard soil extraction methods that target plant-accessible compounds.

Soil greenhouse gas fluxes

In the present study, litter removal significantly changed soil fluxes of all three measured GHGs. In agreement with hypothesis ii, CO_2 fluxes were reduced by 29.9 % in LR plots, and litter-induced contribution to total CO_2 flux ranged from 15.6 to 46.1 %. This is in line with previous studies that have reported a litter-induced contribution of 5–45 % total soil CO_2 flux in temperate forests (Borken and Beese 2005; Bowden et al. 1993;

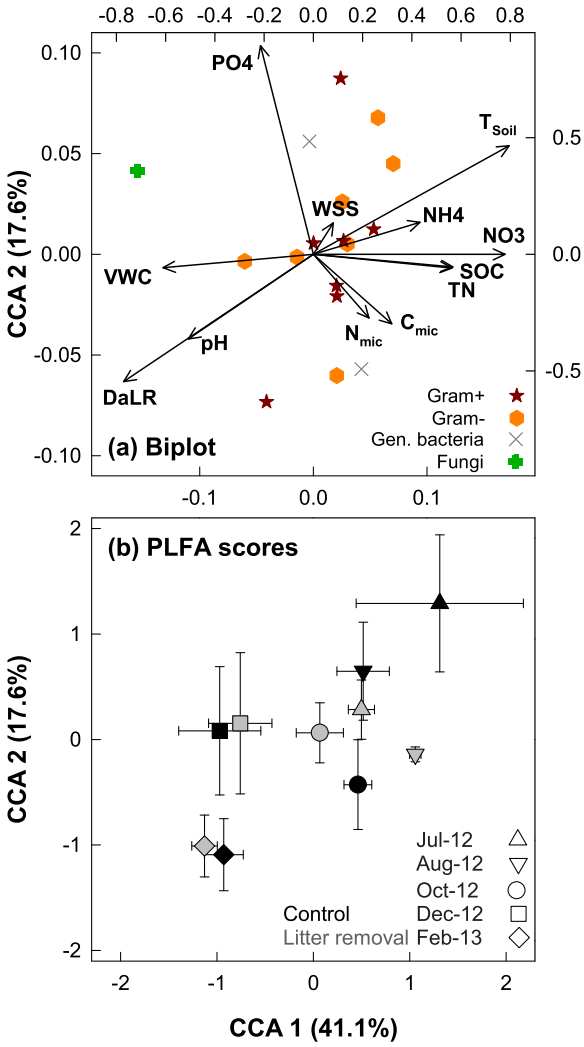

Fig. 4 Influence of soil parameters and time on microbial community composition as determined by canonical correspondence analysis (CCA). **a**, Biplot with microbial groups (gram+, gram-positive bacterial PLFAs; gram-, gram-negative bacterial PLFAs; gen. bacteria, unspecific bacterial PLFAs; fungi, fungal PLFA 18:2ω6,9) and explaining environmental variables as factor loadings (*arrows*). We used relative abundances (%mol) of single PLFA markers as soil microbial community matrix, and soil parameters (pH; VWC, soil volumetric water content; T_{Soil}, soil temperature; SOC, soil organic carbon; TN, total nitrogen; C_{mic}, microbial carbon; N_{mic}, microbial nitrogen; NH4, ammonium-N; NO3, nitrate-N; PO4, phosphate-P; WSS, water-soluble sugars) and time (DaLR, day after litter removal) as constraining variables. **b**, Distribution of samples collected at 5 time points in 2 treatments according to the PLFA species matrix (mean ± 95 % CI, $n = 3$)

Vose and Bolstad 2007). High contributions of the litter layer to total soil CO_2 fluxes can be explained by the active decomposition of litter material, which is rich in easily available C and nutrients. In the present study, the amount of C stored in the litter layer was estimated to be 0.55 kg C m^{-2}, which represents 21 % of the total soil C stock (litter-C + mineral soil-C in 0–5 cm soil depth). Carbon dioxide fluxes of both LR and control plots were closely related to T_{soil}. Temperature sensitivity as expressed by Q_{10} was higher in LR plots, indicating that CO_2 flux from mineral soil was more temperature-sensitive than litter-induced CO_2 flux. Similar results were reported by Creamer et al. (2015) for an Australian native woodland, who reported that the temperature sensitivity of litter-C was lower than that of soil-C. This supports the theory that with decreasing substrate quality, temperature sensitivity of soil CO_2 flux increases because more enzymatic steps are required to break down low-quality organic matter, and each of these steps in turn is temperature sensitive due to microbial enzyme kinetics (Bosatta and Ågren 1999; Fierer et al. 2005; Yuste et al. 2007). However, because we have not tested the temperature sensitivity of litter-induced CO_2 flux alone, we cannot prove this assumption.

Methane fluxes were negative during the entire study period, which indicates constant uptake of atmospheric CH_4 by soils of both treatments. Well-aerated soils of upland forests have been shown to act mostly as CH_4 sinks due to high activity of methanotrophic bacteria that oxidize CH_4 under aerobic conditions to produce energy (Blais et al. 2005; Le Mer and Roger 2001). In our study, litter removal increased average CH_4 uptake by 16.0 %, which corroborates hypothesis iii. We found highest litter-induced contributions to total CH_4 fluxes between November and January, where CH_4 uptake was between 19.9 and 100.9 % higher in LR plots than in control plots. This period was characterized by steadily increasing VWC due to frequent rainfalls. Soil VWC was similar in both treatments at all sampling dates and can therefore not explain different CH_4 fluxes in the two treatments. However, we assume that the wet litter layer itself acted as a barrier against diffusion of atmospheric CH_4 into the soil and, therefore, reduced CH_4 uptake in control plots. This has also been suggested for subtropical forests (Wang et al. 2013) and temperate forests, especially broad-leaved forests like beech (Brumme and Borken 1999). Nevertheless, we cannot test this assumption because we measured only net CH_4 fluxes but not CH_4 diffusion. Furthermore, leachates such as monoterpenes from litter have been described to suppress CH_4 consumption in mineral soils (Amaral and Knowles 1997, 1998), from which we conclude that

litter removal increases CH_4 consumption and that the inhibitory effect of the litter layer might be stronger in the wet season. We found a positive correlation between CH_4 fluxes and VWC in control plots, which indicates lower CH_4 uptake rates (i.e. less negative CH_4 fluxes) at high VWC in the presence of an intact litter layer. If soil VWC is high, soil O_2 levels are low, which can reduce CH_4 oxidation by methanotrophic bacteria and decrease CH_4 uptake rates.

In accordance with hypothesis iv, litter removal decreased average N_2O fluxes by 117.5 % and turned soils from N_2O sources (5.72 μg N_2O-N h^{-1} m^{-2} in controls) to moderate N_2O sinks (−1.00 μg N_2O-N h^{-1} m^{-2} in LR). Nitrous oxide uptake by soils of various ecosystems has frequently been reported and was reviewed by Schlesinger (2013) but has also been challenged as measurement error (Cowan et al. 2014). In the present study, three N_2O emission peaks were measured in control plots between August and October, which all coincided with rapid increases in soil VWC due to heavy rainfalls after dry periods. Interestingly, these peaks only occurred in control plots, although VWC was not different between treatments. It is, however, possible that after rainfall the wet litter layer acted as diffusion barrier for O_2 and created anoxic microsites in control plots where N_2O was produced. Another possible explanation is that increased runoff due to litter removal led to higher local aeration and therefore reduction of N_2O to N_2 in aerobic microsites, although VWC was not lower in LR plots. It is also conceivable that N_2O was produced in the wet litter layer itself, which is rich in C and N to support nitrification and denitrification, and which after rainfalls might contain enough moisture to form anoxic microsites. Dong et al. (1998) reported that 50 % of emitted N_2O in a German beech forest originated from the leaf litter/humus layer. In the present study, we observed high N_2O emissions from both control and LR plots in January 2013. This could be explained by the presence of a thin snow and ice layer that might have acted as diffusion barrier against O_2 and thus created anoxic conditions in both treatments. This was corroborated by low CH_4 consumption rates in control and LR plots at this particular date. Furthermore, although negative soil temperatures in 5 cm depth were not recorded on any of the gas sampling dates, a preceding freeze-thaw event on the soil surface could have led to elevated N_2O fluxes on this date, as has been observed earlier (e.g., van Bochove et al. 2000; Teepe et al. 2001; Wolf et al. 2012; Butterbach-Bahl et al. 2013). We found no correlations between N_2O fluxes and T_{soil} or VWC, which indicates that N_2O formation and consumption was limited by low N content and acidic pH at our study site, as has also been reported for other temperate forests (Butterbach-Bahl et al. 1998; Castro et al. 1992; Hahn et al. 2000).

Soil microbial community composition

Our data suggest an initial transient effect of litter removal on soil microbial abundance and community composition. At the first sampling 1 week after litter removal, C_{mic} as well as PLFAs of gram + and gram- bacteria increased in LR plots, whereas we found no difference between treatments at the consecutive samplings. This immediate increase in bacterial PLFAs could be a consequence of the litter removal at the beginning of the experiment. Although we took great care to completely remove the litter layer, we cannot rule out that some remains of fine debris were left on the LR plots. This remaining fine debris would probably be slightly damaged and also well-aerated because the litter layer on top was removed. Because fragmentation increases litter decomposability (David and Handa 2010; Hassall et al. 1987), this might have led to a flush of available C and nutrients, which could have supported fast-growing bacteria and led to increased concentrations of bacterial PLFAs at the first sampling. In the long term, however, we did not find any influence of litter removal on the contribution of fungi to the soil microbial community, which refutes hypothesis v. This is in line with a study of Brant et al. (2006), which studied the influence of above- and below-ground litter manipulation on soil microorganisms at 3 different sites in the USA and Hungary. They reported no influence of aboveground litter removal after 4, 7 and 13 years, respectively. Similar to our results, Creamer et al. (2015) reported that bacterial community composition analysed by terminal restriction fragment length polymorphism (T-RFLP) was not different in mineral soils compared to mineral soils mixed with pre-incubated eucalyptus litter. In a study that used ^{14}C-labelled leaf litter, Kramer et al. (2010) discovered

that recent (<4 year old) leaf litter made up <10 % microbial-C in mineral soil of a temperate oak forest, whereas greatest inputs to microbial-C originated from roots. Our results corroborate that removal of aboveground litter does not influence microbial community composition of mineral soils within 8 months.

Similar to previous studies, we found a significant influence of seasonality on soil microbial community composition (Kaiser et al. 2010; Koranda et al. 2013; Rasche et al. 2011). From summer to winter, bacterial and fungal PLFA markers increased slightly. CCA analysis of single PLFA composition showed that differences between sampling time points were larger than between treatments. Our results indicate that seasonal differences in microbial community composition seem to be linked to soil pH, T_{soil} and VWC. In a study in an Austrian beech forest similar to our site, Kaiser et al. (2010) also found a significant influence of soil moisture and temperature on soil microbial community composition. This seems plausible, as water availability and temperature are well-known determinants of microbial metabolism. Overall, our data confirm the importance of seasonal changes in temperature, moisture availability and soil nutrient cycling for the composition of microbial communities in temperate forest soils.

Conclusions

The litter layer contributes largely to soil GHG fluxes and influences temperature sensitivity of soil CO_2 fluxes. This should be accounted for in climate change models as litter represents a major component of total C input to soils. Our results suggest that in the short term, the litter layer controls soil GHG fluxes mainly via physical processes and C chemistry and not via nutrient leaching into the mineral soil. Furthermore, our data indicate that nutrient leaching from litter does not determine microbial community composition in the mineral soil in the short term. Our results are relevant for the basic understanding of forest biogeochemical cycles and should be taken into account when assessing GHG budgets in forests.

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