RESEARCH ARTICLE

Fungal and bacterial utilization of organic substrates depends on substrate complexity and N availability

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Abstract

There is growing evidence of a direct relationship between microbial community composition and function, which implies that distinct microbial communities vary in their functional properties. The aim of this study was to determine whether differences in initial substrate utilization between distinct microbial communities are due to the activities of certain microbial groups. We performed a short-term experiment with beech forest soils characterized by three different microbial communities (winter and summer community, and a community from a tree-girdling plot). We incubated these soils with different ^{13}C-labelled substrates with or without inorganic N addition and analyzed microbial substrate utilization by ^{13}C-phospholipid fatty acid (PLFA) analysis. Our results revealed that the fate of labile C (glucose) was similar in the three microbial communities, despite differences in absolute substrate incorporation between the summer and winter community. The active microbial community involved in degradation of complex C substrates (cellulose, plant cell walls), however, differed between girdling and control plots and was strongly affected by inorganic N addition. Enhanced N availability strongly increased fungal degradation of cellulose and plant cell walls. Our results indicate that fungi, at least in the presence of a high N supply, are the main decomposers of polymeric C substrates.

Introduction

Due to the high diversity of soil microorganisms it has long been assumed that there is a functional redundancy between distinct microbial communities from different ecosystems and soil types, which implies that different microbial communities have similar functional properties and similar capacities for degradation of a certain substrate (Andren & Balandreau, 1999; Nannipieri *et al.*, 2003; Wertz *et al.*, 2006). However, this hypothesis has been brought into question by studies which have demonstrated that distinct microbial communities differ in their physiological capacities, indicating a clear relationship between microbial community composition and function (Waldrop & Firestone, 2004; Balser & Firestone, 2005; Paterson *et al.*, 2011).

It is thus currently an important issue in ecosystem ecology to determine the specific role of different groups of soil microorganisms in decomposition processes and to investigate how their activities are differentially influenced by nutrient availability and other environmental factors.

An approach increasingly used during the last decade for monitoring substrate utilization of microbial groups (at a low phylogenetic resolution) is the analysis of carbon isotope ratios of microbial phospholipid fatty acids (PLFAs) combined with stable isotope labelling. In several studies it was observed that labile plant-derived C was predominantly metabolized by Gram-negative bacteria and fungi, whereas Gram-positive bacteria seemed to be involved in the degradation of soil organic matter (SOM; Treonis *et al.*, 2004; Bird *et al.*, 2011). Furthermore,

FEMS MICROBIOLOGY ECOLOGY

degradation of complex and N-poor substrates was mainly ascribed to fungi, whereas bacteria depended on labile C sources (Meidute *et al.*, 2008; Paterson *et al.*, 2008; Paterson *et al.*, 2011). This is consistent with the fact that fungi are generally considered as the main degraders of lignocellulose due to their hyphal growth form, which allows them to redistribute nutrients to nutrient-poor substrates, and their ability to produce extracellular oxidative enzymes (De Boer *et al.*, 2005; Valaskova *et al.*, 2007; Baldrian, 2008).

There is, however, also evidence of functional redundancy between microbial groups, meaning that bacteria and fungi compete for the same substrates (De Boer *et al.*, 2005; Strickland & Rousk, 2010), as reported for labile C substrates (Waldrop & Firestone, 2004; Paterson *et al.*, 2011) as well as complex substrates (Rousk *et al.*, 2008; Strickland & Rousk, 2010). The degree of functional redundancy or functional differences between different microbial groups regarding decomposition of different substrates is, however, still not completely clear, especially in the context of different nutrient requirements of microbial groups and the resulting competitive advantages.

We have recently reported that distinct microbial communities differed in their functional properties (Koranda *et al.*, 2013). Here we focus on the question whether differences in functional properties of microbial communities are related to the activities of certain microbial groups. We hypothesized (1) that distinct microbial communities differ in their utilization of organic substrates and partitioning of substrate-derived C within the microbial community, such that bacteria are more competitive for simple substrates than fungi; and (2) that enhanced inorganic N availability more strongly increases bacterial utilization of C substrates than fungal substrate utilization.

We performed an incubation experiment with beech forest soils characterized by three microbial communities. We collected soils in summer and winter as we expected adaptation of the summer community to high availability of labile C and adaptation of the winter community to more recalcitrant substrates (litter). In summer we also collected soil from a tree-girdling plot in which belowground carbon allocation had been interrupted, promoting the establishment of a more saprotrophic community. We incubated the different soils with a range of ^{13}C-labelled substrates (glucose, protein, microbial cell walls, cellulose, plant cell walls) and additionally enhanced inorganic N supply (in cellulose and plant cell wall treatments). We analyzed respiration of substrate-C as well as the recovery of substrate-C in marker PLFAs specific for certain groups of microorganisms in order to unravel the fate of decomposed substrates within the soil microbial communities.

Materials and methods

Soil

The soil for the incubation experiment originated from a 65-year-old beech forest (*Fagus sylvatica*) about 40 km southwest of Vienna (48°07′N 16°03′E, 510 m above sea level). Soil was classified as Dystric Cambisol over flysh. Soil characteristics (pH, moisture, C/N ratio) are given in the Supporting Information, Table S1. Soil was collected in February 2008 (winter community) and June 2008 (summer community and community from girdling plots). Girdling of beech trees had been performed in May 2006 by removal of the bark over 10-cm sections around the circumference of the stems. The experimental setup in the field, as well as microbial processes and microbial communities, have been described in detail by Kaiser *et al.* (2010, 2011) and Rasche *et al.* (2010). Soil was stored at 4 °C (winter) and 12 °C (summer) for 2 weeks until the start of the incubation experiment. Half of the winter soil was transferred to 12 °C for equilibration 3 days before the incubation. Soil characteristics (C and N pools, functional microbial communities) of soils collected in winter and in summer from control and from girdled plots have been described in Koranda *et al.* (2013) and the microbial community composition (determined from phospholipid fatty acids) is given in Table 1. Although the relative abundance of major microbial groups did not differ significantly between the soils, a principal component analysis (PCA) calculated from all PLFA abundances revealed distinct microbial community composition of soils collected in winter and in summer from control and from girdled plots (data not shown).

Substrates

Five ^{13}C-labelled substrates differing in complexity and C and N content were used for the incubation experiment: glucose, protein, microbial cell walls, cellulose and plant cell walls, containing 20 atom% ^{13}C, except for cellulose (16 atom%) and protein (98 atom%). Glucose (99 atom% ^{13}C, from Sigma) and cellulose (97 atom% ^{13}C, from IsoLifeBV) were diluted with the respective unlabelled substances and algal protein extract (98 atom % ^{13}C, N content *c.* 60%, from Sigma) was applied undiluted.

^{13}C-labelled microbial cell walls were prepared as follows: Two bacterial species (*Pectobacterium carotovorum and Verrucomicrobium spinosum*) and one fungal species (*Aspergillus nidulans*) were grown on ^{13}C-glucose (20 atom% ^{13}C). Growth conditions have been described by Keiblinger *et al.* (2010). Microbial biomass was dried and then resuspended in NaCl-solution. After mechanical

Table 1. Characterization of microbial communities in soils collected in winter and in summer from control plots and girdled plots. Values are means ($n = 3$), SE in brackets

	Gram$^-$ bacteria (mol%)	Gram$^+$ bacteria (mol%)	Fungi (mol%)	Total PLFAs (nmol g^{-1} DW)
Winter	31.4 (0.6)	18.7 (1.4)	11.9 (0.4)	554 (136)
Summer – Control	30.6 (0.2)	18.1 (1.5)	13.0 (0.6)	619 (90)
Summer – Girdling	30.9 (2.1)	17.2 (0.4)	11.3 (1.0)	429 (45)

destruction of cell walls by ultrasonic treatment and bead-beating, residues were repeatedly extracted with NaCl-solution, water, methanol/chloroform (5 : 3), hexane and pure water to remove all labile cell constituents. The remaining residues were dried, homogenized (ball mill) and stored frozen.

^{13}C-labelled plant cell walls were prepared as follows: ^{13}C-labelled wheat roots (IsoLiveBV) and unlabelled, dried wheat roots were mixed and finely ground and homogenized in a ball mill. The material was then incubated with an α-amylase solution to remove starch (Richter *et al.*, 2009) and further extracted repeatedly with methanol/chloroform/water (12 : 5 : 3) to remove other labile substances. Plant cell walls contained 0.83% N after removal of labile substances (probably in cell wall proteins).

Experimental setup

Soils were sieved (5 mm) and root fragments were removed with forceps. The respective substrate (1 mg substrate g^{-1} soil of glucose and protein, corresponding to *c.* 130 and 115% of microbial biomass C, respectively, and 4 mg g^{-1} soil of the other substrates) was amended to each soil in a dry form. A subset of the summer soils (from control and girdling plots) amended with either cellulose or plant cell walls, was also amended with inorganic N (3 mg NH_4NO_3 g^{-1} soil). We added N to these treatments to test the effects of increased N availability on the degradation of polymeric C substrates (one of them containing lignin), assuming microbial N limitation in summer. For practical reasons (number of samples) we decided to add N to incubations of two substrates only.

Incubation of soils (22 g) was performed in a microcosm system with four replicates for each substrate and soil (Inselsbacher *et al.*, 2009). Controls without added substrate were prepared for each soil (2 × 4 replicates per soil). Microcosms were loosely sealed with moist cotton wool and incubated in the dark for 2 days (glucose and protein incubations) or 6 days (microbial cell walls, cellulose and plant cell wall incubations). Incubation times were chosen to include the peak of $^{13}CO_2$ release following the addition of labile substrates. For incubations of complex substrates, we chose an incubation time of 6 days, which should ensure comparability of the treatments and avoid internal redistribution of ^{13}C within the microbial communities. The anticipated durations of incubations had been determined in a pre-experiment. Incubation temperature was 12 °C; additionally soils from the winter community were incubated at 4 °C. The fate of C substrate in the winter community did not differ significantly between the two incubation temperatures. For better comparability of respiration rates, only the results of 12 °C winter incubations are presented here. At the end of the incubation period, microcosms were destructively harvested for determination of microbial phospholipid fatty acids.

Microbial respiration

Microbial respiration rates were measured at 5 time points during the incubation period. Prior to each gas sampling, incubation tubes were sealed at the bottom, cotton wool was removed and instead, polypropylene tubes closed by airtight rubber caps were mounted on the incubation tubes (Inselsbacher *et al.*, 2009). Fifteen millilitres of headspace gas were sampled by syringe immediately after closing the tubes and replaced by 15 mL of air (ambient CO_2 concentration). A second gas sample was taken after 30 min. Concentration and carbon isotope ratio of CO_2 (relative to VPD) was determined via a GasBench II interfaced to continuous-flow isotope ratio mass spectrometry (IRMS; Delta V Advantage, Thermo Fisher, Germany). Respiration from substrate was calculated according to the following equation:

$$R_{\text{substrate}} = \text{APE}^{13}\text{C}_{\text{resp}} / \text{APE}^{13}\text{C}_{\text{substrate}} * R_{\text{total}}$$

where $\text{APE}^{13}\text{C}_{\text{resp}}$ and $\text{APE}^{13}\text{C}_{\text{substrate}}$ is atom% excess ^{13}C in respiration and substrates, respectively, and R_{total} is total respiration.

Phospholipid fatty acids

PLFAs in soils were extracted with a mixture of methanol, chloroform and citrate buffer (2 : 1 : 0.8, v/v/v), then separated from neutral lipids on silica columns and finally subjected to alkaline methanolysis (see Koranda *et al.*, 2011 for details). Blanks of substrates without soils were

treated similarly to verify that substrates did not contain PLFAs. Dried fatty acid methyl esters were re-dissolved in isooctane and concentrations and carbon isotope ratios of PLFAs were determined by a Trace Ultra GC (Thermo Fisher) interfaced with an IRMS (Delta V Advantage, Thermo Fisher) via a combustion unit (GC combustion II/TC, Thermo Fisher). A mixture of fatty acid methyl esters (FAMEs; Supelco, nr. 47080-U and 47885-U) was used as a qualitative standard. An internal standard (19 : 0) was used for calculation of FAME concentrations, as well as for correction of $\delta^{13}C$ values. $\delta^{13}C$ values of PLFAs were also corrected for $\delta^{13}C$ values of the C added during methanolysis. We used the sum of the fatty acids i15:0, a15:0, i16:0, i17:0, a17:0 as an indicator of Gram-positive bacteria, the sum of 16:1ω9, 16:1ω7, 16:1ω5, 18:1ω7, 18:1ω5, cy17:0, cy19:0, cy18:0 as an indicator of Gram-negative bacteria, and the sum of fatty acids 15:0 and 17:0 as an indicator for bacteria in general (Zelles, 1997; Leckie, 2005). The PLFA 16:1ω5 has also been reported as a marker for arbuscular mycorrhizal fungi (Olsson, 1999). However, as in beech forest soils ectomycorrhiza is the dominant form of mycorrhiza and moreover, mycorrhizal fungi had probably decayed in our system due to absence of plant roots, 16:1ω5 is most likely an indicator of Gram-negative bacteria in this soil. PLFAs 18:2ω6,9, 18:2ω3,6,9 and 18:1ω9 were used as fungal markers. The PLFA 20:4ω6,9,12,15 has been proposed as a marker for protozoa (Lechevalier, 1977) or fungi (Stressler *et al.*, 2013).

Incorporation of substrate-derived C in PLFAs was calculated according to the following equation:

$$C_{Inc} = APE^{13}C_{PLFAs}/APE^{13}C_{substrate} * C_{PLFAs}$$

where $APE^{13}C_{PLFAs}$ and $APE^{13}C_{substrate}$ are atom% excess ^{13}C in PLFAs and substrate, respectively, and C_{PLFAs} is the C content in PLFAs.

Statistics

Data were transformed prior to analysis to achieve normality and homogeneity of variances (logarithmic transformation was applied for absolute process rates, square root transformation for percentage values). Differences in respiration and substrate incorporation between the three soils were assessed using one-way ANOVA and Tukey's *post-hoc* test. Effects of N addition to incubations of complex C substrates were estimated by Student's *t*-test. ANOSIM (analysis of similarity) was applied for determination of differences in the distribution of substrate C within microbial communities. We used the programs PRIMER 6 for ANOSIM and STATISTICA 6.0 for all other analyses.

Results

Microbial substrate utilization

In all incubations, a peak in substrate respiration shortly after the beginning of the incubation period followed by a decline towards the end of the first day was observed, most likely because also complex substrates contained a small number of monomers (Fig. 1). Respiration of complex substrates (microbial cell walls, cellulose and plant cell walls) increased from the 3rd day until the end of the incubation period (except for cellulose incubations without inorganic N).

Microbial communities in soils collected in winter or summer differed significantly in the cumulative respiration of labile substrates (Fig. 1, Table 2). Whereas the summer community more intensively respired C from glucose compared with the winter community, a higher respiration of added protein by the winter community was observed. Cumulative respiration of cellulose and plant cell walls was significantly enhanced by the addition of inorganic N ($P < 0.001$). At high N availability, soils from girdled plots exhibited higher respiration of complex C substrates than soils from control plots ($P < 0.001$).

At the end of the incubation period the summer community had incorporated three times as much C from glucose as the winter community (Table 2). Substrate incorporation into PLFAs was significantly enhanced by N addition in incubations of cellulose ($P < 0.05$ and $P < 0.001$ in control soil and soil from girdled plots, respectively) and slightly increased in incubations of plant cell walls ($P < 0.1$ in soils from girdled plots). The microbial community in soil from girdled plots incorporated more C from cellulose compared with the community in control plots at high N availability ($P < 0.05$).

Differences in microbial biomass size between soils collected in winter, summer and from girdled plots, however, need to be considered when estimating differences in microbial substrate incorporation and respiration (microbial biomass C, determined by chloroform-fumigation-method, was higher in summer than in winter, and lowest in soil from girdled plots (37.0, 31.7 and 24.2 μmol g^{-1} DW, respectively). If values were calculated per unit biomass C, microbial decomposition of complex C substrates (cellulose and plant cell walls) was generally higher in soils from girdled plots than control plots at both low and high N availability (except for substrate incorporation from plant cell walls; data not shown).

The ratio of substrate-derived C in PLFAs to respired substrate C was lowest in glucose incubations of the winter community and highest in incubations of microbial cell walls, as well as plant cell wall incubations of the

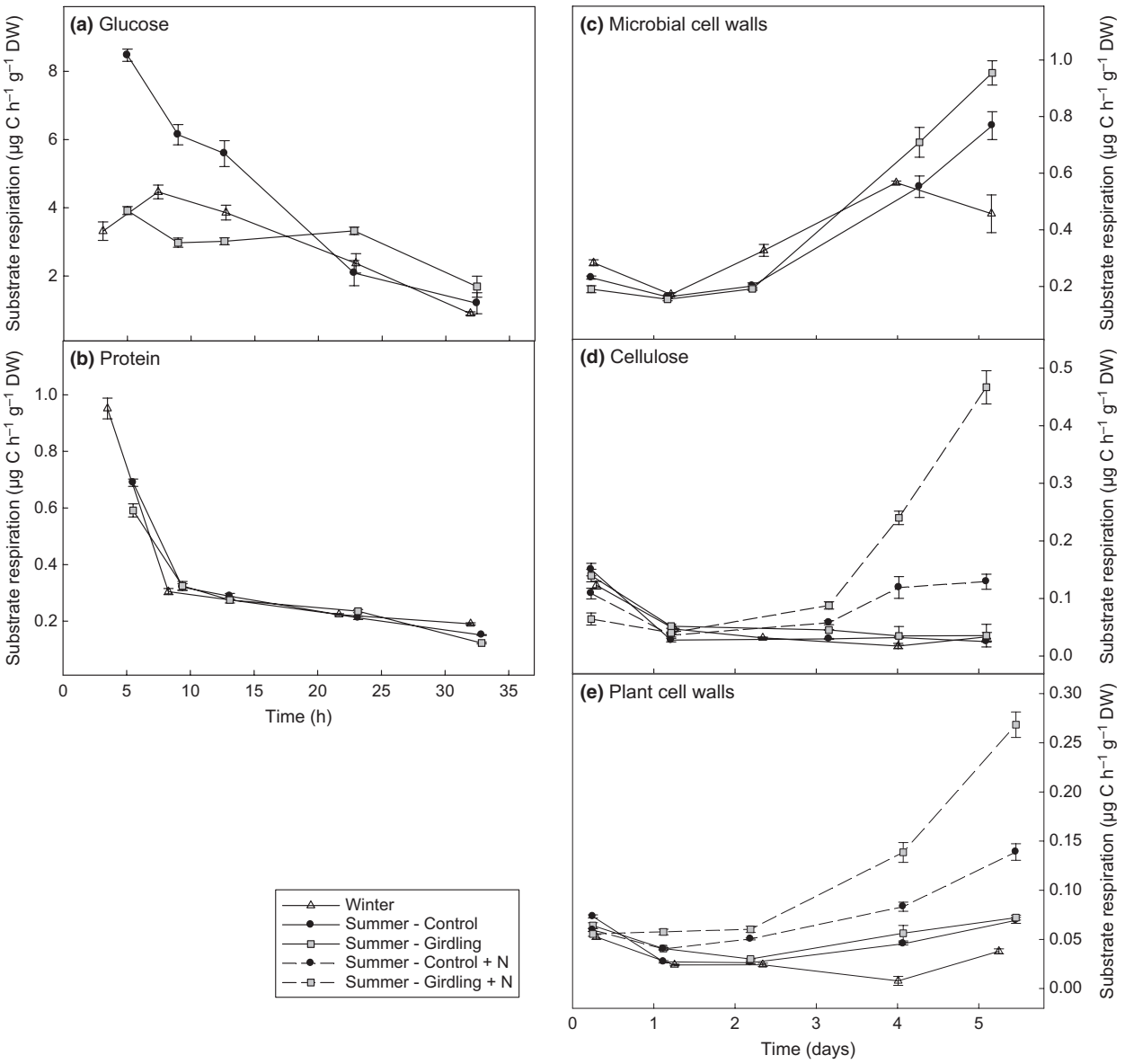

Fig. 1. Substrate respiration in soils collected in winter (triangles), in summer from control plots (circles) and from girdled plots (squares), incubated with five organic substrates with or without inorganic N addition. Values are means ± SE (n = 4).

winter community (Table 2). Inorganic N addition to incubations of cellulose significantly increased this ratio ($P < 0.001$ and $P < 0.05$ in soils from control and girdled plots, respectively), whereas in incubations of plant cell walls the ratio was decreased ($P < 0.001$ in soil from control plots) or was not affected by N addition.

Distribution of incorporated substrate C within the microbial community

A principal component analysis from relative ^{13}C incorporation into single PLFAs (in percent of ^{13}C in total PLFAs) revealed that the distribution of substrate-derived C within the microbial community was mainly determined by the type of substrate added (Fig. 2a; ANOSIM $P = 0.001$, $R = 0.836$). Differences between microbial communities in the distribution of substrate C were only observed for incubations of cellulose, and for plant cell walls and cellulose incubations with added N. Addition of inorganic N to complex C substrates strongly altered the fate of substrate C within the microbial community compared with incubations without N. Factor loadings on PCA-axis 1 were highest for fungal marker PLFAs and two marker PLFAs for Gram-negative bacteria

Table 2. Cumulative respired substrate-derived C and substrate-derived C in PLFAs at harvest in incubations of soils collected in winter, in summer from control plots and girdled plots, incubated for 2 days (with labile substrates, i.e. glucose and protein) or 6 days (with complex substrates).

	Respired substrate-derived C (μg g^{-1} DW)			Substrate derived C in PLFAs (μg g^{-1} DW)			Substrate derived C in PLFAs/ Respired substrate-derived C		
	W	S	G	W	S	G	W	S	G
Glucose	98.0[a]	137.0[b]	98.5[a]	3.33[a]	10.05[b]	7.47[b]	0.03[a]	0.08[b]	0.08[b]
Protein	11.1[a]	10.0[b]	9.5[b]	1.94	1.59	1.21	0.18	0.15	0.13
Microbial cell walls	48.5	46.0	53.0	15.16	15.42	13.42	0.31	0.34	0.26
Cellulose	5.6	5.7	7.2	0.55	0.37	0.75	0.10	0.07	0.11
Cellulose + N	n.d.	10.0[a]	18.3[b]	n.d.	1.42[a]	3.10[b]	n.d.	0.14	0.16
Plant cell walls	3.3[a]	5.6[b]	6.4[b]	0.87	0.72	0.40	0.26[a]	0.13[b]	0.06[b]
Plant cell walls + N	n.d.	9.1[a]	13.9[b]	n.d.	0.64	0.93	n.d.	0.07	0.07

n.d., not determined.

Mean values: $n = 4$ (respiration) or $n = 3$ (PLFAs). Significant differences (Tukey's *post-hoc* test; $P < 0.05$) are indicated by different letters. Data based on Koranda *et al.* (2013)

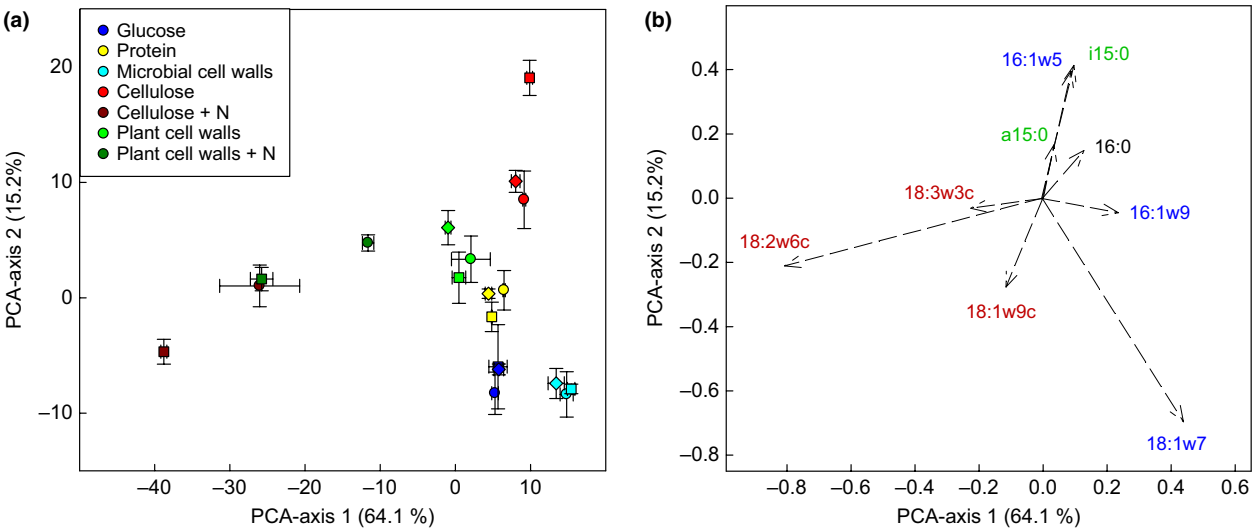

Fig. 2. Results of a PCA (principal component analysis) calculated from relative ^{13}C incorporation into single PLFAs (in% of ^{13}C incorporation in total PLFAs). (a) Plot of samplings describing differences in the fate of substrate C between incubations of soils collected in winter (diamonds), in summer from control plots (circles) and from girdled plots (squares), incubated with five different organic substrates with or without inorganic N addition. (b) Factor plot indicating contribution of variables (PLFAs). Fungal marker PLFAs are written in red, markers for $Gram^-$ bacteria in blue, and markers for $Gram^+$ bacteria in green. Values are means ± SE ($n = 3$).

(Fig. 2b). Bacterial markers had the highest loadings on PCA-axis 2.

The pattern of the distribution of substrate C within the microbial communities is further illustrated by the substrate incorporation into marker PLFAs for different microbial groups (in percent of total incorporated substrate C; Fig. 3). At low N availability the highest proportion of total substrate-derived C was generally detected in marker PLFAs for Gram-negative bacteria, with especially high values being found in incubations of microbial cell walls (*c.* 47%), followed by cellulose (33–41%) and glucose incubations (*c.* 36%). Markers for Gram-positive bacteria incorporated the highest proportion of substrate C in cellulose incubations (between 20% and 27%). The highest substrate incorporation into fungal markers was detected in incubations of glucose (20–23% of total incorporated C) and plant cell walls (17–20%). In winter, fungal markers incorporated a significantly higher proportion of protein than in summer (19% compared with 15.3%, respectively).

Inorganic N addition not only increased microbial utilization of complex C substrates (Table 2), but also altered the proportional substrate utilization of microbial groups (Fig. 3). Whereas the proportion of substrate C in fungal markers was significantly enhanced by N addition to cellulose and plant cell wall incubations, the proportion

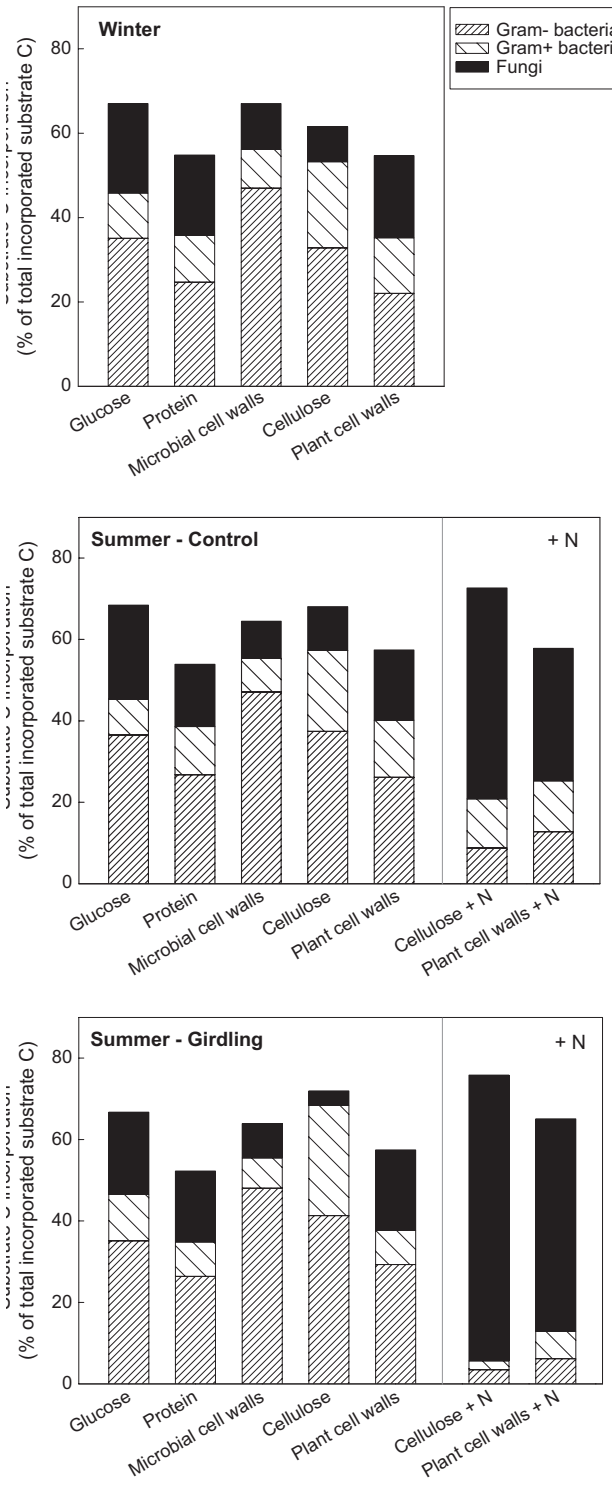

Fig. 3. Relative substrate C incorporation (in% of total incorporated substrate C) into marker PLFAs for microbial groups. The difference to 100% is due to ^{13}C incorporation of ubiquitous or not specified PLFAs. Soils collected in winter, in summer from control plots and from girdled plots were incubated with five organic substrates with or without inorganic N addition. Values are means of three.

of substrate incorporation in Gram-negative bacteria declined (as well as substrate incorporation in Gram-positive bacteria in cellulose incubations of soil from girdled plots). The increase in fungal substrate incorporation by enhanced N availability was significantly stronger in soil from girdled plots than from control plots ($P < 0.001$ and $P < 0.05$, respectively), substrate C in fungal markers accounting for 70% and 52% of total recovered substrate C in N-amended cellulose and plant cell wall incubations of soil from girdled plots, respectively. In cellulose incubations of control soil the observed decline in relative bacterial substrate incorporation by N addition was mainly due to a 20-fold increase in absolute fungal substrate incorporation but absolute bacterial substrate utilization was not significantly changed (Table 3). In cellulose incubations of soil from girdled plots, however, a dramatic increase in fungal activity by N addition (85-fold increase in ^{13}C incorporation) was accompanied by a significant decline in absolute substrate incorporation of both Gram-positive and Gram-negative bacteria. ^{13}C incorporation of Gram-negative bacteria also tended to decline at high N availability in incubations of plant cell walls.

Table 3. Incorporation of substrate-derived C into marker PLFAs for different microbial groups. Soils collected in winter, in summer from control plots and from girdled plots were incubated with five organic substrates with or without inorganic N addition. Values are means of three; SE are given in brackets

	Gram$^-$ bacteria	Gram$^+$ bacteria	Fungi
Substrate-derived C in PLFAs (μg g^{-1} DW)			
Winter			
Glucose	1.17 (0.12)	0.36 (0.04)	0.70 (0.06)
Protein	0.47 (0.05)	0.21 (0.03)	0.37 (0.04)
Microbial cell walls	7.09 (1.32)	1.34 (0.06)	1.64 (0.31)
Cellulose	0.18 (0.03)	0.11 (0.02)	0.05 (0.01)
Plant cell walls	0.19 (0.02)	0.11 (0.02)	0.17 (0.03)
Summer – Control			
Glucose	3.64 (0.49)	0.93 (0.33)	2.30 (0.30)
Protein	0.42 (0.02)	0.19 (0.04)	0.24 (0.01)
Microbial cell walls	7.24 (0.36)	1.30 (0.31)	1.40 (0.13)
Cellulose	0.13 (0.02)	0.08 (0.04)	0.04 (0.00)
Cellulose + N	0.12 (0.00)	0.16 (0.04)	0.75 (0.21)
Plant cell walls	0.20 (0.08)	0.10 (0.00)	0.12 (0.01)
Plant cell walls + N	0.08 (0.03)	0.08 (0.03)	0.21 (0.08)
Summer – Girdling			
Glucose	2.61 (0.05)	0.87 (0.33)	1.49 (0.14)
Protein	0.32 (0.04)	0.10 (0.01)	0.21 (0.03)
Microbial cell walls	6.49 (1.60)	0.98 (0.14)	1.12 (0.19)
Cellulose	0.31 (0.01)	0.21 (0.05)	0.03 (0.00)
Cellulose + N	0.11 (0.01)	0.07 (0.01)	2.18 (0.18)
Plant cell walls	0.12 (0.03)	0.04 (0.02)	0.08 (0.01)
Plant cell walls + N	0.06 (0.01)	0.06 (0.02)	0.48 (0.04)

^{13}C incorporation into specific phospholipid fatty acids in incubations of different substrates is described in detail in the Supporting Information, Figs S1 and S2, Data S1).

In contrast to the clear differences in the active microbial community between incubations of different substrates and the strong effects of inorganic N addition on microbial activity, the composition of the total microbial community varied only slightly (Table S2).

Discussion

In this study we investigated differences in the utilization of organic substrates between distinct microbial communities as well as differences in the fate of substrate-derived C (i.e. substrate incorporation by microbial groups). We observed that utilization of added substrates by different microbial groups (bacteria and fungi) depended on the type of added substrate and was strongly influenced by inorganic N availability.

Microbial utilization of glucose was higher in soil collected in summer than in winter (probably reflecting adaptation of the summer community to high availability of labile C supplied by plants via belowground C allocation; Table 2) but the distribution of substrate C within the microbial community was similar in both seasons (Figs 2 and 3). This is in line with results from other studies which demonstrated that labile C substrates are equally metabolized by a large range of microbial species (Waldrop & Firestone, 2004; Paterson *et al.*, 2011), predominantly by Gram-negative bacteria and fungi (Treonis *et al.*, 2004; Bird *et al.*, 2011). The low ratio of substrate C in PLFAs over respired substrate C in incubations of glucose, which indicates microbial N limitation, may result from substrate C being stored in neutral lipids (of fungi) instead of being used for growth, as reported in other studies (Baath, 2003; Rinnan & Baath, 2009). Functional differences between summer and winter communities in utilization of another labile substrate, protein (Table 2), seemed to be linked to fungal activity, as indicated by higher fungal substrate incorporation in winter soil (Fig. 3). Unlike protein, microbial cell walls were predominantly utilized by Gram-negative bacteria (which also showed a very high substrate use efficiency for this substrate), suggesting that they either have a unique set of enzymes for chitin and/or peptidoglycan decomposition or that they are especially efficient in taking up the products of decomposition (i.e. are efficient cheaters).

Degradation of complex C substrates was higher in soils from girdled plots than from control plots (Table 2), possibly indicating microbial adaptation to high amounts of dead fine root biomass in girdled plots 3 years after girdling. In cellulose incubations, as well as cellulose and plant cell wall incubations with inorganic N additions, microbial communities from girdled and control plots also exhibited a differing pattern of substrate incorporation of microbial groups (Figs 2 and 3), probably reflecting enhanced activity of saprotrophic microbes specialized on degradation of polymeric C substrates in soil from girdled plots. Functional differences between distinct microbial communities in the degradation of recalcitrant substrates, such as plant litter, which were linked to differences in the distribution of substrates within the microbial communities, have been described previously (Waldrop & Firestone, 2004; Paterson *et al.*, 2011).

In most studies, fungi were reported to be the main decomposers of lignocellulose (De Boer *et al.*, 2005; Paterson *et al.*, 2008). This has, to a large extent, been confirmed by our findings. However, our results revealed that the composition and activity of the microbial community involved in the decomposition of polymeric C substrates was strongly dependent on nutrient availability. At low N availability a large proportion of substrate C from cellulose was incorporated in bacterial markers, especially in soil from girdled plots, whereas fungal incorporation of this substrate into PLFAs was low (Fig. 3, Table 3). The capacity of different bacterial taxa for degradation of cellulose has been reported in other studies (Perestelo *et al.*, 1996; De Boer *et al.*, 2005; Vargas-Garcia *et al.*, 2007; Goldfarb *et al.*, 2011). Bacterial utilization of cellulose, however, should be interpreted with caution. First, bacterial cellulose degradation is probably facilitated by the finely ground form of cellulose applied in our experiment, which makes it more easily accessible for bacteria. Secondly, in cellulose incubations without added N, most substrate C was respired in the initial phase of the incubation, which suggests that bacteria mainly utilized labile compounds contained in the cellulose (e.g., oligomeric sugars). Actual cellulose degradation by bacteria is hence questionable. Longer incubation times would have probably been needed to assess bacterial participation in cellulose decomposition.

Contrary to bacteria, fungi more intensively utilized plant cell walls than cellulose (Fig. 3), probably due to the lignin content of plant cell walls, as well as N contained in cell wall proteins.

If inorganic N was added to cellulose and plant cell walls, fungal decomposition activity of polymeric C substrates strongly increased (Fig. 3, Table 3). N addition also significantly enhanced the ratio of incorporated substrate C over respired substrate C (Table 2) in incubations of cellulose, but not of plant cell walls, which suggests strong N limitation of fungi in cellulose incubations without inorganic N. The low amount of substrate C in fungal marker PLFAs in cellulose incubations without added N may thus underestimate total fungal substrate incorporation, as substrate C from cellulose may

be used for synthesis of storage compounds instead of growth at low N availability (Baath, 2003). This also implies a low production of extracellular enzymes under conditions of N limitation. Addition of inorganic N alleviated fungal N limitation, resulting in strongly enhanced decomposition rates of complex C substrates, increased respiration rates and increased fungal growth, i.e. substrate incorporation in PLFAs. This positive effect of N addition on fungal activity is remarkable, as fungi are generally assumed to have lower N demands than bacteria, due to their higher biomass C : N ratio (Keiblinger *et al.*, 2010; Strickland & Rousk, 2010). However, stimulation of fungal growth or activity by enhanced N availability has also been found in other studies (Rousk & Baath, 2007; Boberg *et al.*, 2008; Meidute *et al.*, 2008; Fontaine *et al.*, 2011), which applied lower N loads than in our study. The often observed fungal dominance in the decomposition of N-poor plant litter hence seems to a great extent to be due to the hyphal growth form, which enables fungi, apart from the penetration of plant cell walls, to import N from N-rich soil horizons (e.g. from degradation of SOM; Frey *et al.*, 2003; Strickland & Rousk, 2010; Fontaine *et al.*, 2011).

Bacterial incorporation of polymeric C substrates, on the other hand, tended to be reduced by N addition (Fig. 3, Table 3). Negative responses of Gram-negative bacteria to N fertilization, especially in the abundance of the marker PLFA 16:1ω5, have also been reported in a previous field study (Weand *et al.*, 2010).

Our results on microbial utilization of polymeric C substrates corroborated in part findings by others. Rousk *et al.* (2008) observed that fungi and bacteria may compete for the same complex substrates, indicating functional redundancy between microbial groups with respect to decomposition processes. In our short-term study, however, mainly fungi were responsible for decomposition of cellulose and plant cell walls, but bacterial decomposition of such substrates at longer incubation times might have been possible. Changes in N availability strongly influenced competitive abilities of microbial groups, fungi outcompeting bacteria at high N availability. In addition, at sufficient N supply the overall decomposition rates increased substantially, indicating that fungi are probably more efficient decomposers of complex C substrates compared to bacteria.

In summary, our results revealed that differences in utilization of a labile C substrate (glucose) between microbial communities (summer and winter community) were not due to the activities of a certain microbial group but instead reflected differences in substrate utilization by a large part of the microbial community. This utilization pattern for labile substrates contradicts our initial hypothesis that distinct microbial communities differ in their utilization of organic substrates and in partitioning of substrate-derived C within the microbial community. Higher degradation of polymeric C substrates (cellulose and plant cell walls) in soils from girdled plots than from control plots, however, was related to an altered composition of the active microbial community in girdled plots.

Decomposition of complex C substrates was markedly increased by enhanced inorganic N availability, with strongly divergent effects on different microbial groups. Contrary to our hypothesis, N addition strongly enhanced fungal decomposition of cellulose and plant cell walls, whereas bacterial utilization of these substrates was not significantly changed or was even reduced. Although the results from this short-term experiment cannot be extrapolated to the field, our results indicate that fungi, at least under high N supply, are the main decomposers of polymeric C substrates.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. ^{13}C incorporation into PLFAs in soils collected in winter (triangles), in summer from control plots (circles) and from girdled plots (squares), incubated with five organic substrates with or without inorganic N addition. Values are means ± SE (n = 3).

Fig. S2. ^{13}C incorporation into PLFAs (absolute values) in soils collected in winter (triangles), in summer from control plots (circles) and from girdled plots (squares), incubated with five organic substrates with or without inorganic N addition. Values are means ± SE (n = 3).

Table S1. Characteristics of soils collected in winter and in summer from control and girdling plots.

Table S2. Microbial community composition (relative abundance of microbial groups in mol%) at harvest in incubations of soils collected in winter, in summer from control plots and from girdled plots, which were incubated with five organic substrates, with or without inorganic N addition.

Data S1. ^{13}C incorporation into specific phospholipid fatty acids.