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DISSERTATION

Titel der Dissertation

„Effects of resource availability and microbial community composition on microbial decomposition processes in beech forest soil“

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I. INTRODUCTION AND OUTLINE

Photosynthetic CO_2 fixation by plants and decomposition of dead biomass by soil microbes are the two major biotic processes forming the terrestrial C cycle (Chapin et al., 2002). Decomposition of soil organic matter (SOM) is performed by soil microbes (bacteria and fungi), which meet their energy needs, as well as their demands for carbon and nutrients from these degradation processes (Adl, 2003). Decomposition rates of SOM are regulated by various factors (Fig. 1), including:

1. Abiotic factors (soil temperature and moisture, pH,...)
2. Substrate quality and availability of labile C and nutrients
3. Composition of the soil microbial community

The influence of soil temperature and moisture on microbial decomposition processes of SOM has been increasingly discussed during the last years in the light of ongoing global change (Conant et al., 2011; Davidson and Janssens, 2006). It is expected that rising temperature will promote mineralization of soil organic C, especially at higher latitudes (Carrillo et al., 2011; Knorr et al., 2005; Zimmermann and Bird, 2012).

Another factor strongly influencing microbial decomposition rates is the quality of organic substrates. For example, it has been demonstrated that decomposition rates of litter from different plant species vary significantly (Bray et al., 2012; Laganiere et al., 2010; Szanser et al., 2011), depending on the chemical composition of the different litter types. Chemical properties which may influence substrate degradability by microbes are the size of the molecules, the regularity of the structure, the type of chemical bonds and finally the content of nitrogen and other nutrients (Chapin et al., 2002). The content of lignin and N, as well as the lignin:N ratio of substrates have been proved as good predictors of litter decomposition rates (Hobbie, 2005; Melillo et al., 1982; Wang et al., 2004).

Microbial decomposition processes have often been assumed to be N-limited as extracellular enzymes synthesized by microbes are N-rich compounds (Allison, 2005; Allison and Vitousek, 2005; Hernandez and Hobbie, 2010). It has, however, been demonstrated that increased inorganic N availability may not only accelerate microbial decomposition of litter or SOM but also exert negative effects on decomposition rates, depending on the type of substrates and the stage of decomposition (Berg and Matzner, 1997; Hobbie, 2005; Lavoie et al., 2011). While degradation of moderately labile substances (such as polysaccharides) by cellulolytic enzymes is often enhanced by increased N availability, decomposition of recalcitrant substrates (such as humus or

lignin) by oxidative enzymes may be reduced (Carreiro et al., 2000; Geisseler and Horwath, 2009; Sinsabaugh et al., 2000).

Apart from N availability, the availability of labile C sources may also influence microbial decomposition of SOM. Frequently, an increase in C mineralization after addition of labile C sources could be observed, an effect termed 'priming effect' (Blagodatskaya and Kuzyakov, 2008; Kuzyakov et al., 2000). This may reflect microbial activation by the pulse of labile C (alleviating microbial C limitation), resulting in enhanced microbial turnover ('apparent priming') (Blagodatskaya and Kuzyakov, 2008; De Nobili et al., 2001). It may also reflect enhanced degradation of N-rich SOM for acquisition of additional N ('microbial N mining') (Fontaine et al., 2003).

Such a priming effect of microbial decomposition of SOM can also be observed in the vicinity of plant roots ('rhizosphere priming') (Bottner et al., 1999; Cheng and Coleman, 1990; Kuzyakov, 2002), as root exudation leads to high availability of labile C in the rhizosphere (Grayston et al., 1997; Jones et al., 2009), which promotes microbial biomass growth and turnover (Butler et al., 2004; Phillips et al., 2011; Priha et al., 1999b). Consequently, C and N cycling is often accelerated in the rhizosphere compared to bulk soil (Norton and Firestone, 1996; Priha et al., 1999a), which is also reflected in the pattern of extracellular enzyme activities (Phillips et al., 2011; Renella et al., 2007).

Apart from effects of resource availability on microbial physiology and activity, alterations in substrate availability may also affect microbial decomposition processes indirectly via influences on microbial community composition. As different microbial species differ in their demands for C and nutrients, as well as substrate preference (Degens, 1999; Gusewell and Gessner, 2009), changes in substrate supply often induce shifts in the composition of soil microbial communities (Eilers et al., 2010; Fierer et al., 2012; Griffiths et al., 1999; Waldrop et al., 2004). Alterations in community structure, in turn, may strongly influence microbial decomposition of SOM since certain classes of extracellular enzymes are produced by certain groups of microorganisms. This is most likely the case for 'phylogenetically narrow processes', i.e. processes performed by a relatively small number of microbial species, such as polyphenol degradation, while other processes, such as mineralization of labile C sources or protein depolymerization, are probably less sensitive to microbial community changes (Balser and Firestone, 2005; Schimel et al., 2005; Waldrop and Firestone, 2004). The link between microbial community composition and microbial processes is, however, still not completely clear. Several studies have observed functional differences between distinct microbial communities, e.g.

communities from different ecosystems, which suggests a direct relationship between microbial community composition and function (Balser and Firestone, 2005; Paterson et al., 2011; Strickland et al., 2009; Waldrop and Firestone, 2004). But there is also evidence of functional redundancy between different groups of microbes, as demonstrated by similar capacities of bacteria and fungi for degradation of labile substrates (Paterson et al., 2011; Waldrop and Firestone, 2004) and also complex substrates (Rousk et al., 2008; Strickland and Rousk, 2010).

This thesis presents results of the RELIS-project (**Re**source **li**mitation of microbial decomposition of **s**oil organic matter), which aimed at gaining more insight into the relationship between resource availability, microbial community structure and microbial functions. In this project we performed a two-year-seasonal experiment in a temperate beech forest, combined with a tree girdling experiment, in which we removed the bark from trees in order to interrupt plant C exudation into the soil (Fig. 2 and 3).

Results of the field experiment investigating the effects of seasonal variation in plant belowground C allocation on microbial decomposition processes, community composition and microbial biomass dynamics are presented in the appendix of this thesis. These two publications have been written by my colleague, Christina Kaiser. I performed a great part of the field work and chemical analyses. In this study we observed that seasonal variation in substrate availability as well as abiotic factors caused a seasonal pattern in microbial processes, which was also related to microbial community changes. The reduction in plant belowground C allocation by tree girdling markedly altered the soil microbial community structure, with strong effects on microbial process rates.

As the influence of plant belowground allocation on soil microbes is naturally most pronounced in the vicinity of plant roots, we performed an additional sub-project focusing on microbial processes in the rhizosphere compared to bulk soil. Publication 1 of this thesis elucidates how microbial processes and community structure in the rhizosphere are affected by plant C exudation and by the reduction of plant influence following tree girdling. Our results revealed a positive rhizosphere effect on gross N mineralization and hydrolytic enzyme activites but a negative effect on oxidative enzyme activities and nitrification rates.

The second part of our project consisted of an incubation experiment performed with soil from the beech forest study site, which we incubated with various organic substrates and

inorganic N. By this experiment we aimed at further investigating the link between microbial functions and community composition. We aimed at elucidating if the seasonal or experimentally induced changes in microbial processes, which we had observed in the field, were related to alterations in functional capacities of microbial communities.

In publication 2 we demonstrate that distinct microbial communities in soils collected at different seasons and from girdled plots varied in their functional properties. The winter community exhibited a higher capacity for degradation of complex C substrates than the summer community, while utilization of labile C substrates was lower in winter than in summer. The saprotrophic community in girdled plots exhibited a higher capacity for degradation of complex C substrates than the community in control plots if additional N was provided.

Publication 3 focuses on the question whether differences in substrate utilization between distinct microbial communities were due to activities of certain microbial groups. We observed that fungal and bacterial groups may be redundant with respect to degradation of complex C substrates, but that they were differentially influenced by changes in nutrient availability.

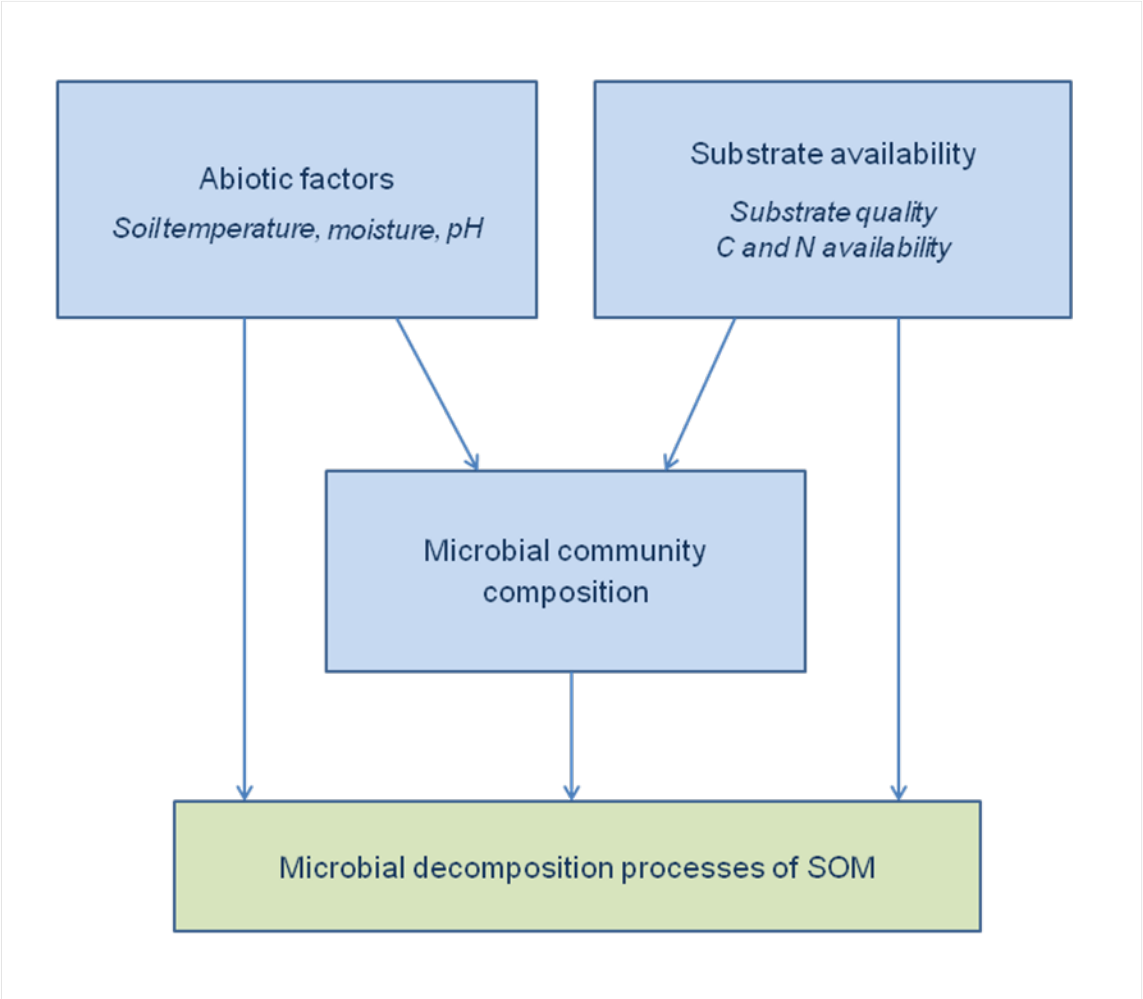

Fig. 1. Schematic representation of factors regulating microbial decomposition processes

Fig. 2. Beech forest study site in summer and winter

Fig. 3. Girdled beech tree

Photos: F. Hadacek, B. Wild, B. Kitzler

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II. Microbial processes and community composition in the rhizosphere of European beech – The influence of plant C exudates

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Microbial processes and community composition in the rhizosphere of European beech – The influence of plant C exudates

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ABSTRACT

Plant roots strongly influence C and N availability in the rhizosphere via rhizodeposition and uptake of nutrients. This study aimed at investigating the effect of resource availability on microbial processes and community structure in the rhizosphere. We analyzed C and N availability, as well as microbial processes and microbial community composition in rhizosphere soil of European beech and compared it to the bulk soil. Additionally, we performed a girdling experiment in order to disrupt root exudation into the soil. By this novel approach we were able to demonstrate that enhanced resource availability positively affected N mineralization and hydrolytic enzyme activities in the rhizosphere, but negatively affected nitrification rates and oxidative enzyme activities, which are involved in the degradation of soil organic matter. Both rhizosphere effects on N mineralization and oxidative enzyme activities disappeared in the girdling treatment. Microbial community structure in the rhizosphere, assessed by phospholipid fatty acid analysis, differed only slightly from bulk soil but was markedly altered by the girdling treatment, indicating additional effects of the girdling treatment beyond the reduction of root exudation. Differences in oxidative enzyme activities and nitrification rates between rhizosphere soil and bulk soil, however, suggest considerable differences in the (functional) microbial community composition.

1. Introduction

It has long been recognized that plant roots exert a large influence on the soil environment and its microflora and that these interactions in turn have considerable impact on plant growth (Lambers et al., 2009; Pinton et al., 2007). The term 'rhizosphere' has been coined to describe the soil adjacent to and influenced by plant roots, a zone of usually high microbial activity, clearly distinct from bulk soil with regard to availability of nutrients, water and oxygen, in pH and redox potential (Hinsinger et al., 2009). The main reason for the high abundance of microorganisms in the vicinity of plant roots is rhizodeposition, which may account for as much as 25% of belowground allocated C (or 10% of net fixed C) (Jones et al., 2009; Uren, 2007; Whipps, 1990) and comprises water soluble root exudates such as sugars, amino acids, organic acids and hormones, as well as mucilage, enzymes, sloughed root cells and C allocated to root-associated symbionts (e.g. mycorrhiza) (Dennis et al., 2010; Grayston et al., 1997; Uren, 2007). Due to the high C/N ratio of rhizodeposits and due to plant nutrient uptake, the rhizosphere environment is characterized by a surplus of easily available C and as a result a strong nutrient limitation (Kuzyakov, 2002).

Positive as well as negative effects of plant roots on microbial growth and microbial decomposition processes have been observed, which is probably due to differing soil N and C availabilities, as well as differences in the investigated soil types or soil horizons (Kuzyakov, 2002). Although competition for water and N may even result in reduced microbial activity in the vicinity of roots (Wang and Bakken, 1997), plant-derived labile C sources often stimulate microbial growth and are rapidly metabolized (Butler et al., 2004). Several studies have shown that microbial activation by plant C exudates and N limitation in the rhizosphere may cause enhanced microbial decomposition of SOM (Bird et al., in press; Bottner et al., 1999; Cheng and Coleman, 1990), presumably because microbes use energy and C supplied by easily assimilable compounds to synthesize enzymes to hydrolyze, in turn, more complex SOM to acquire additional N (Blagodatskaya and

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Kuzyakov, 2008; Kuzyakov, 2002). Such enhanced decomposition of recalcitrant compounds after the addition of easily assimilable compounds or regular polymers (e.g. cellulose) has been termed "rhizosphere priming" (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2003; Kuzyakov, 2010). In some studies, however, the observed increase in C mineralization following the addition of labile C compounds was rather attributed to higher microbial turnover rates and breakdown of microbial C pools than to enhanced depolymerisation of SOM (De Nobili et al., 2001; Weintraub et al., 2007).

It is widely known that the rhizosphere is a zone of high microbial turnover and activity, and several studies investigated the influence of plant roots on specific microbial processes, such as extracellular enzyme activities (Kaiser et al., 2010b; Weintraub et al., 2007), N mineralization (Norton and Firestone, 1996), nitrification and denitrification (Priha et al., 1999a, 1999b). Differences in microbial community structure between rhizosphere and bulk soil have already been analyzed by means of phospholipid fatty acid profiles (Butler et al., 2003; Paterson et al., 2007; Steer and Harris, 2000), or by molecular techniques (Marilley and Aragno, 1999), but few studies have been reported so far that link microbial community structure to microbial processes.

The goal of the present study was to explore how rhizosphere processes and microbial community composition are affected by resource availability. Our hypothesis was that root exudates facilitate the establishment of a distinct microbial community in the rhizosphere, and that an interruption of plant C exudation would cause the rhizosphere community to become more similar to bulk soil community. We further hypothesized that differences in resource availability between rhizosphere and bulk soil would result in different microbial processes, either as an effect of distinct microbial communities or as a direct priming effect on microbial decomposition processes by root exudates. In order to experimentally alter the resource availability we reduced plant C exudation by tree girdling, a method which has successfully been applied to disrupt belowground carbon allocation in trees (Högberg et al., 2001; Kaiser et al., 2010b). The combination of a comprehensive analysis of microbial processes, community composition, and C and N availability in the rhizosphere on the one hand and an experimental approach to reduce the plant influence on the soil microbial community on the other hand, is novel and allows to dissect the effect of resource availability and community composition on microbial processes.

2. Material and methods

2.1. Study site

Our study site was located in a 70 year old beech forest (*Fagus sylvatica*) about 40 km southwest from Vienna (48°07' N 16°03' E, 510 m a.s.l.). Tree density at the study site is 710 stems ha^{-1}, with a canopy height of 26 m and mean diameter at breast height of trees of 21.5 cm. The mean air temperature at the study site is approximately 8 °C and the mean annual precipitation is 730 mm. Soil type is a Dystric Cambisol over flysh (pH in $CaCl_2$ 4.5–5.1) with an organic carbon content of 7.45% and total nitrogen content of 0.48% in the A horizon.

2.2. Experimental setup

Three circular girdling plots (15 m in diameter) and 6 control plots (5 m × 5 m) were established at the study site. Girdling of trees was performed in May 2008 by removal of the bark over 10 cm sections around the circumference of the stems at about 1.5 m above ground. Understory vegetation was removed from girdling and control plots.

2.3. Soil sampling

The first sampling was performed prior to girdling in May 2008 (bulk soil only), the main sampling was performed in September 2008. Six replicate samples of mineral soil were taken from control and girdling plots, respectively (2 replicates per plot in case of girdling plots, taken only from the inner 5 m diameter zone of the girdling plots). Each replicate consisted of 5 subsamples (soil cores of 7 cm diameter and 14.5 cm depth). Rhizosphere soil and roots were separated from bulk soil by hand: the soil closely adhering to the roots (up to 2.5 mm around the root) was considered as rhizosphere soil. Bulk samples were sieved (<2 mm). Bulk and rhizosphere samples were stored at 4 °C.

2.4. Soil extracts, C and N content

Two aliquots of fresh soil (2 g) were extracted with 20 ml of water and 1 M KCl, respectively. After adding $HgCl_2$-solution (to a final concentration of 10 μM) to water extracts, extracts were stored at −20 °C. Subsamples of soil were oven-dried, weighed and ground for determination of C and N content by elemental analysis (EA 1110, Carlo Erba Instruments, Milan, Italy).

2.5. Inorganic N, DON, DOC and microbial biomass

NO_3^- and NH_4^+ were determined from water extracts by chemically suppressed ion-chromatography (Dionex HPAEC on a AS11 column for anions and Dionex HPCEC on a CS16 column for cations) (Kaiser et al., 2005). Organic C and total dissolved N were analyzed in water extracts by a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu). Dissolved organic N was calculated by subtracting inorganic N from total dissolved N.

2.6. Gross N mineralization and gross nitrification

Gross N mineralization and gross nitrification were assessed using the pool dilution technique (Kaiser et al., 2005; Myrold and Tiedje, 1986): 500 μl of $^{15}NH_4Cl$ or $K^{15}NO_3$ (0.25 mM, 10 atom% ^{15}N), respectively, were applied to 2 subsamples (2 g) of fresh soil, then incubated for 4 h and 24 h and finally extracted with 15 ml of 2 M KCl. For determination of mineralization ^{15}N values of NH_3 diffused into acid traps were analyzed by continuous-flow isotope ratio mass spectrometry (IRMS) using an elemental analyzer coupled to a gas IRMS system (DeltaPLUS, Finnigan MAT, Bremen, Germany). $^{15}N/^{14}N$ ratios of NO_3^- were determined according to the protocol from Stange et al. (2007). In the SpinMAS system NO_3^- in the soil extract was reacted to NO by the addition of acidic $V(III)Cl_3$ solution. The produced NO was purged from H_2O and CO_2 with a cryotrap and ^{15}N abundance of the NO was analyzed in a quadrupole mass spectrometer (GAM 400, InProcess Instruments GmbH, Bremen, Germany). Rates of gross N mineralization and gross nitrification were calculated according to the following equations:

$$m = (A_t - A_0)/t^* (\ln(APE_0/APE_t)/\ln(A_t/A_0))$$

where m is gross mineralization, A_t is the NH_4^+–N pool after time t, A_0 is the initial NH_4^+–N pool, APE (atom percent excess) is at $\%^{15}N_{sample}$–at$\%^{15}N_{natural\ abundance}$

$$m = (N_t - N_0)/t^* (\ln(APE_0/APE_t)/\ln(N_t/N_0))$$

where n is gross nitrification, N_t is the NO_3^-–N pool after time t, N_0 is the initial NO_3^-–N pool.

2.7. Extracellular enzyme activities

Enzyme activities were assayed by microplate fluorimetric and photometric assays: 1 g of fresh, sieved soil was suspended in 100 ml sodium acetate buffer (100 mM, pH 5.5) and ultrasonicated at low-energy for 2 min. Peptidase and phosphatase were measured fluorimetrically (Marx et al., 2001; Saiya-Cork et al., 2002): 200 μl of soil suspension and 50 μl of substrate (0.5–1 mM L-leucine 7-amido-4-methyl coumarin and 2 mM 4-methylumbelliferyl-phosphate, respectively) were pipetted into black microtiter plates (3 analytical replicates). After incubation in the dark for 140 min fluorescence was measured at 450 nm emission and 365 nm excitation (Tecan Infinite M200 fluorimeter). Aminomethylcoumarin (AMC) was used as a calibration standard for peptidase whereas Methylumbelliferyl was used for calibration of phosphatase.

Phenoloxidase and peroxidase were measured according to standard methods (Sinsabaugh et al., 1999), with small modifications: Subsamples of the soil suspension were mixed with an L-3,4-dihydroxyphenylalanin (DOPA) solution (20 mM DOPA, 1:1), shaken for 10 min and centrifuged. Aliquotes were pipetted into transparent microtiter plates with 6 analytical replicates per sample. Half of the wells additionally received 10 μl of a 0.3% H_2O_2 solution for measurement of peroxidase activity. Absorption at 450 nm was measured at the starting time point and after an incubation time of 20 h. Enzyme activity was calculated from the difference in absorption divided by an extinction-factor of 0.62, which was determined in a pre-experiment.

2.8. PLFAs

Phospholipid fatty acids were analyzed using a modified procedure described by (Frostegard et al., 1991). Fresh soil samples (2 g) were extracted overnight with a mixture of citrate buffer (0.15 M, pH 4.0 with NaOH), chloroform and methanol at a ratio of 0.8: 1: 2 (v/v/v). Samples were centrifuged and the supernatant was transferred to new vials. After addition of chloroform and citrate buffer for separation of the phases, the organic phase was removed and dried under a stream of dry N_2. Lipids were redissolved in chloroform and neutral lipids were separated from phospholipids on silica columns (Supelco) by subsequent elution with chloroform, acetone and methanol. The methanol–fraction was dried under N_2. Phospholipids were subsequently converted to fatty acid methyl esters by alkaline methanolysis. After adding 100 μL of a solution of methyl-nonadecanoate (200 μg mL^{-1}) as an internal standard, phospholipids were dissolved in 1 mL of methanolic 0.2 M KOH and 1 mL of methanol-toluene (1:1) and incubated for 15 min at 37°, then mixed with 2 mL of water, 300 μL of acetic acid and 2 mL of hexane-chloroform (4:1). The hexane–phase was removed and dried under N_2. Dried FAMEs were redissolved in isooctane and analyzed by gas chromatography (HP G1530A) on a DB23 column (Agilent, Vienna, Austria). A mixture of bacterial FAMEs (Supelco, Vienna, Austria) was used as a qualitative standard. Concentrations of single FAMEs were calculated using the internal standard (19:0) peak as a reference. PLFAs were designated following standard nomenclature, i.e. the number before the colon refers to the number of C atoms, the number after the colon indicates the number of double bonds followed by the position of the double bonds from the methyl end (ω). The prefixes i, a, and cy indicate iso- and anteiso-branching, and cyclopropyl-groups, respectively. We used the sum of the fatty acids i15:0, a15:0, i16:0, i17:0, a17:0 as indicator of Gram-positive bacteria, the sum of 16:1ω9, 16:1ω7, 18:1ω7, 18:1ω5, cy17:0, cy19:0, cy18:0 as indicator of Gram-negative bacteria, and all these together with 17:0, 17:1ω6, 17:1ω7 as a measure for total bacteria (Leckie, 2005; Zelles, 1997, 1998). The quantity of 18:2ω6,9 was used as an indicator of fungal biomass. 18:2ω6,9 is also common in plant tissue. However, it has recently been shown that the contribution of plants to this biomarker was negligible (Kaiser et al., 2010a).

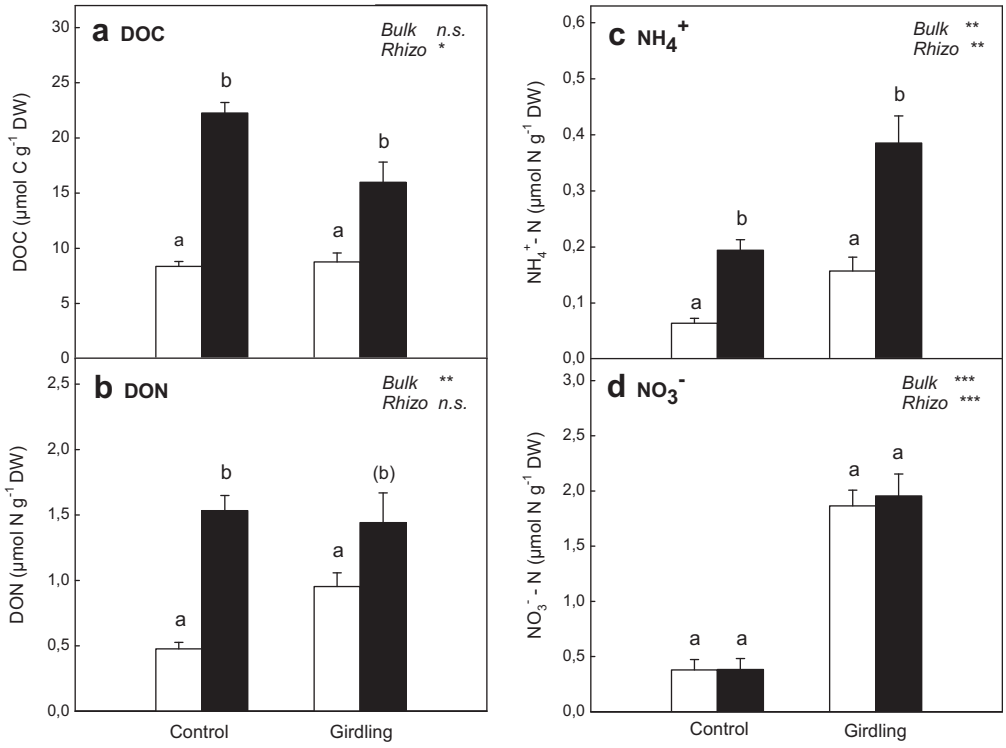

Fig. 1. Concentrations of (a) DOC, (b) DON, (c) NH_4^+ and (d) NO_3^- in bulk soil (open bars) and rhizosphere soil (black bars) of control plots and girdling plots. Significant differences between rhizosphere and bulk soil are indicated by different letters ($p < 0.05$; letters in brackets indicate significance level of $p < 0.1$). Significance of differences between control plots and girdling plots for bulk soil and rhizosphere soil, respectively, is indicated by *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$) or n.s. (not significant). Error bars represent standard errors ($n = 6$).

2.9. Statistical analysis

Data were transformed to their natural logarithms in order to achieve normality and homogeneity of variances before analysis. Differences between rhizosphere and bulk soil, as well as differences between treatments were assessed using student's *t*-test. Microbial community composition was analyzed by a principal component analysis (PCA) of the relative abundances of PLFAs. Differences between microbial communities were estimated by an analysis of similarity (ANOSIM) using a distance matrix of Euclidian distances. ANOSIM is a procedure which generates an R test statistic from distances between and within groups of samplings. The higher the R-value, the greater is the difference between groups. Pearson correlations were used for relating microbial processes to abundances of PLFAs, and for relating PCA-axes to C and N pools and microbial processes, respectively. Univariate statistical analyses were performed using Statistica 6, multivariate analyses were performed by Primer 6.

3. Results

3.1. C and N availability

Concentrations of dissolved organic C and N were significantly higher in the rhizosphere than in bulk soil (Fig. 1a and b). Girdling significantly reduced DOC concentrations in the rhizosphere, whereas no effect of girdling on rhizospheric DON concentrations and a significant increase in DON concentrations in bulk soil was observed. Concentrations of NH_4^+ were significantly enhanced in rhizosphere soil compared to bulk soil (Fig. 1c), while no differences between rhizosphere and bulk soil were found for NO_3^- (Fig. 1d). Concentrations of inorganic N were markedly increased by girdling, especially concentrations of NO_3^-, suggesting a stronger effect of the girdling treatment on N than on C availability.

3.2. Microbial processes

Gross N mineralization rates were significantly higher in the rhizosphere of control plots compared to bulk soil (Fig. 2a), while this rhizosphere effect was not observed in girdling plots. Gross nitrification rates, on the contrary, were lower in the rhizosphere compared to bulk soil (Fig. 2b). Nitrification accounted for only 10% of gross N mineralization in the rhizosphere, compared to 33% in bulk soil and 60% in bulk soil of girdling plots. Unfortunately, nitrification rates in the rhizosphere of girdling plots could not be measured due to problems during mass spectrometry.

Extracellular enzyme activities separated into two groups: Peptidase and phosphatase activities (Fig. 3a and b) were increased in the rhizosphere of control plots and these enzyme activities were enhanced by the girdling treatment in bulk soil only. Oxidative enzyme activities (Fig. 3c and d), in contrast, were reduced in the rhizosphere compared to bulk soil in control but not in girdling plots.

3.3. Microbial community composition

Surprisingly, a principal component analysis (PCA) and analysis of similarity (ANOSIM) of the relative abundances of phospholipid fatty acids (PLFAs) revealed that microbial community composition of the rhizosphere was not clearly distinct from bulk soil community composition in control plots (Fig. 4, Table 1). Girdling, however, lead to a marked shift in the rhizosphere community composition. A correlation of the results of the PCA with C and N pools and microbial processes exhibited that the first PCA-axis, separating the rhizosphere of girdling plots from the rhizosphere of control plots and from bulk soils was negatively correlated to NH_4^+ concentrations ($p < 0.05$). The second PCA-axis, mainly separating bulk soil from rhizosphere soil of control plots, showed a positive relationship to DOC and DON concentrations ($p < 0.05$), as well as correlations to oxidative enzyme activities and nitrification ($p < 0.1$).

The results of the PCA are further supported by the absolute abundances of PLFAs specific for different microbial groups (Fig. 5b–d). No significant differences between rhizosphere and bulk soil of control plots were observed for any of the microbial groups. Girdling reduced fungal PLFAs in the rhizosphere (Fig. 5b). Marker fatty acids of both Gram-positive and Gram-negative bacteria were enhanced by girdling in bulk soil (Fig. 5c and d), while a significant decrease in rhizosphere abundance of Gram-positive bacteria was found. These patterns were reflected in the total abundance of PLFAs, used to estimate total microbial biomass (Fig. 5a), as we observed no significant differences between rhizosphere and bulk soil in control plots, but an increase in total PLFAs in bulk soil and a decrease in the rhizosphere by the girdling treatment.

Correlations between the abundances of selected group-specific PLFA–markers and microbial processes (Table 2) revealed that gross mineralization rates, as well as phosphatase and peptidase activities, which are all phylogenetically broad processes, i.e. processes related to a wide range of microbial taxa, were positively correlated to the abundance of marker fatty acids of various microbial groups and also to ubiquitous fatty acids. Gross nitrification rates and oxidative enzyme activities, on the contrary, showed mainly negative correlations to both bacterial and fungal marker fatty acids.

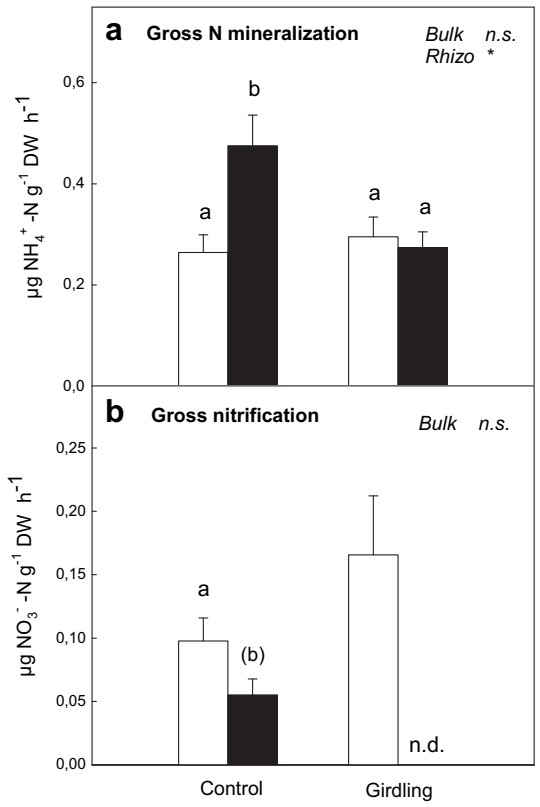

Fig. 2. (a) Gross N mineralization rates and (b) gross nitrification rates measured in bulk soil (open bars) and rhizosphere soil (black bars) of control plots and girdling plots. Significant differences between rhizosphere and bulk soil are indicated by different letters ($p < 0.05$; letters in brackets indicate significance level of $p < 0.1$). Significance of differences between control plots and girdling plots for bulk soil and rhizosphere soil, respectively, is indicated by * ($p < 0.05$) or n.s. (not significant). Error bars represent standard errors ($n = 6$). n.d. means 'not determined'.

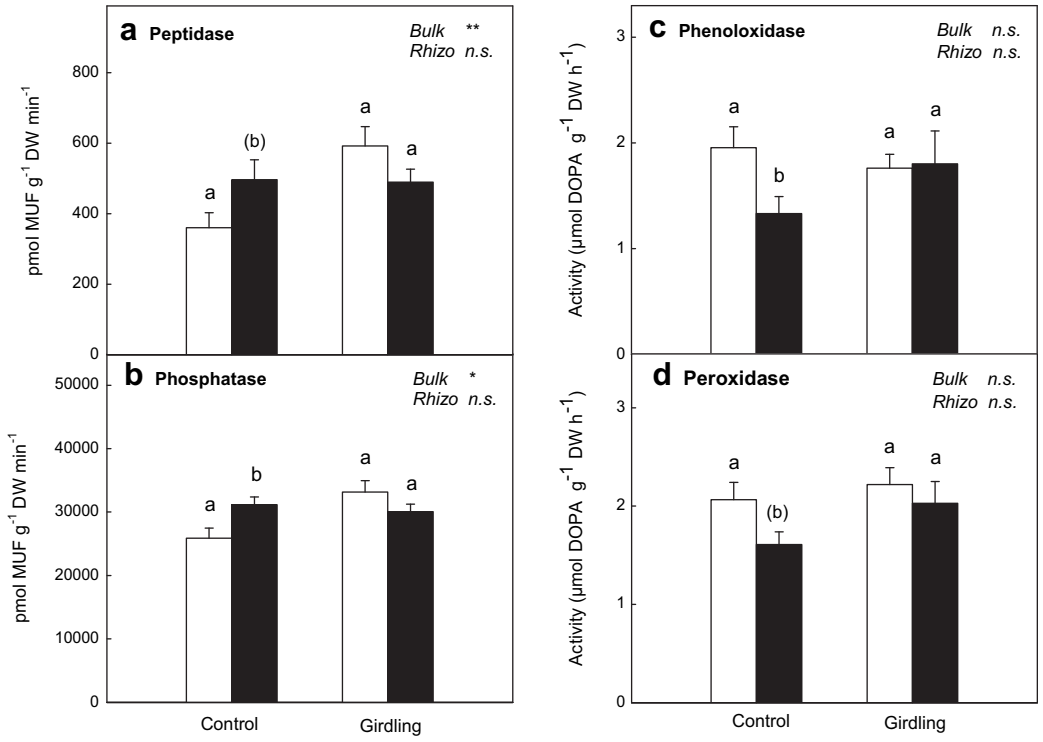

Fig. 3. Extracellular enzyme activities ((a) peptidase, (b) phosphatase, (c) phenoloxidase and (d) peroxidase) measured in bulk soil (open bars) and rhizosphere soil (black bars) of control plots and girdling plots. Significant differences between rhizosphere and bulk soil are indicated by different letters ($p < 0.05$; letters in brackets indicate significance level of $p < 0.1$). Significance of differences between control plots and girdling plots for bulk soil and rhizosphere soil, respectively, is indicated by ** ($p < 0.01$), * ($p < 0.05$) or n.s. (not significant). Error bars represent standard errors ($n = 6$).

4. Discussion

Despite the relatively small percentage that the rhizosphere occupies of the total soil volume, rhizosphere processes may be more important than bulk soil processes for soil functioning and may play a vital role in ecosystem nutrient cycling (Badalucco and Nannipieri, 2007). In this study we investigated microbial processes in the rhizosphere of European beech. We expected that differences in resource availability between rhizosphere and bulk soil would result in distinct microbial processes and community structure, and that these differences would be reduced by an interruption of belowground carbon allocation.

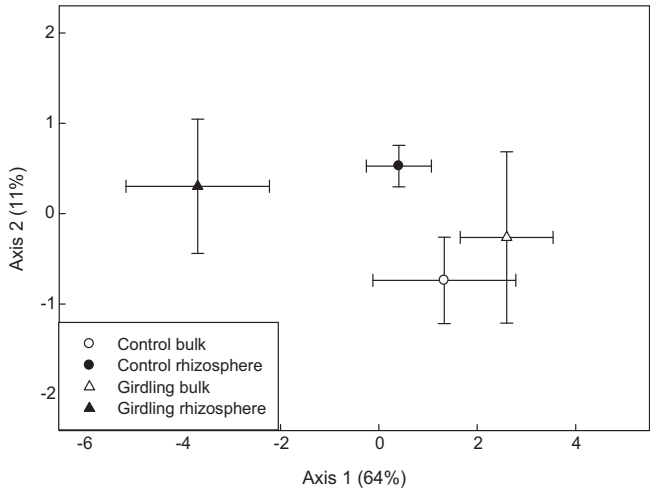

Fig. 4. Microbial community composition described by a principal component analysis of the relative abundances of PLFAs. Error bars represent standard errors ($n = 6$).

The rhizosphere soil was characterized by markedly higher C and N availability compared to bulk soil. Girdling significantly decreased concentrations of DOC in the rhizosphere (Fig. 1a). Decreased concentrations of DOC were also found in previous girdling experiments in bulk soils (Kaiser et al., 2010b; Weintraub et al., 2007). Although DOC concentrations in the rhizosphere were reduced in girdled plots, they were still higher than concentrations in the bulk soil, maybe due to the fact that DOC may also originate from degradation of SOM (Ekberg et al., 2007; Kalbitz et al., 2000). It should be kept in mind, however, that our data do not allow us to distinguish between enhanced production and decreased uptake of DOC or DON, for example by plant roots or microbes. The significant increase in concentration of DON in bulk soil of girdling plots corresponds to increased peptidase activities, indicating that the measured potential enzyme activities can be linked to the concentration of enzymatic products.

Concentrations of NH_4^+ were significantly enhanced in the rhizosphere, while no differences were found for NO_3^-, which is most likely due to the high mobility of NO_3^- in soil (Fig. 1c and d). The marked increase in concentrations of inorganic N by the

Table 1
Results of ANOSIM describing differences between microbial communities by analysis of PLFA-abundances.

	R-value*	Significance Level %
Control bulk–rhizo	0.083	19.7
Girdling bulk–rhizo	0.445	1.9
Bulk control–girdling	−0.084	81.7
Rhizo control–girdling	0.193	9.5

* R-value indicates the degree of separation of two communities.

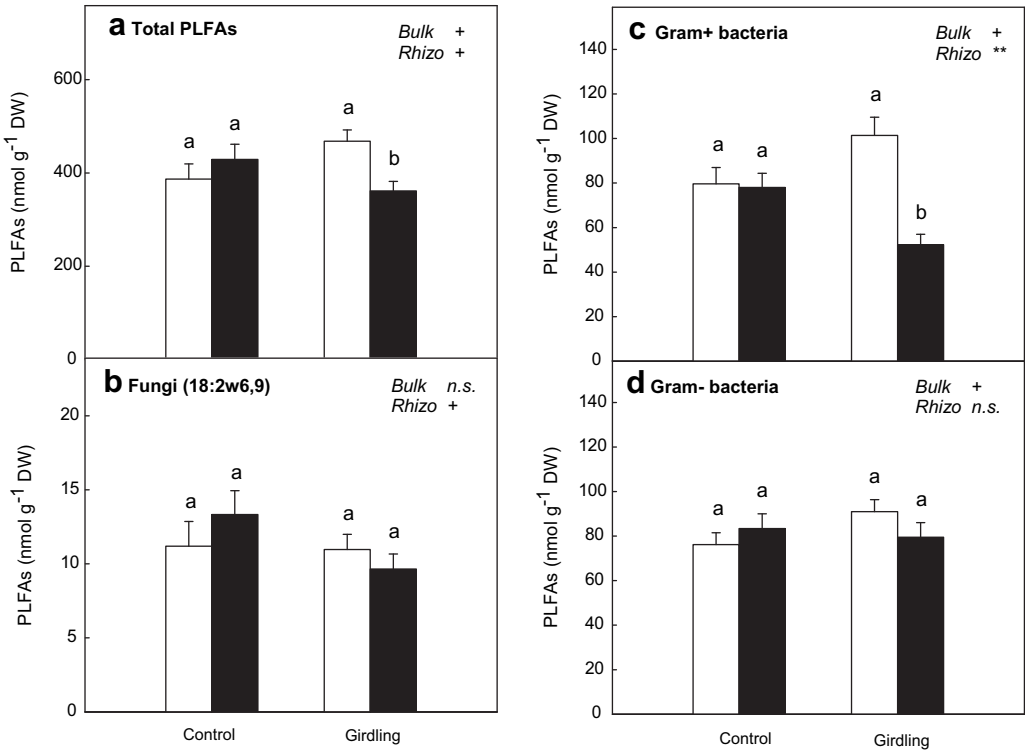

Fig. 5. Concentrations of (a) total PLFAs and marker PLFAs for (b) fungi, (c) Gram-positive bacteria and (d) Gram-negative bacteria in bulk soil (open bars) and rhizosphere soil (black bars) of control plots and girdling plots. Significant differences ($p < 0.05$) between rhizosphere and bulk soil are indicated by different letters. Significance of differences between control plots and girdling plots for bulk soil and rhizosphere soil, respectively, is indicated by ** ($p < 0.01$), +($p < 0.1$) or n.s. (not significant). Error bars represent standard errors ($n = 6$).

girdling treatment, which has already been observed in a previous experiment (Kaiser et al., 2010b) is probably an effect of reduced plant and mycorrhizal N uptake, since uptake of nutrients is an energy requiring process and root C reserves were exhausted by the girdling treatment (data not shown).

High resource availability in the rhizosphere was reflected in the pattern of microbial processes: N mineralization rates were strongly increased in the rhizosphere (Fig. 2a), as already reported for rhizosphere soils of trees (Norton and Firestone, 1996). The fact that the marked difference in N mineralization rates between rhizosphere soil and bulk soil of control plots disappeared in the girdling treatment, clearly points to microbial activation by supply of labile C ("rhizosphere priming") as suggested previously (Blagodatskaya and Kuzyakov, 2008; De Nobili et al., 2001; Weintraub et al., 2007).

Nitrification rates, on the contrary, tended to be lower in the rhizosphere (Fig. 2b). This could be interpreted as an effect of competition between nitrifiers and plant roots for NH_4^+. However, as nitrification rates were measured in incubated soils after separation from plant roots, it is also possible that low nitrification rates are the result of the dominance of fast growing heterotrophic bacteria in the rhizosphere.

Regarding extracellular enzyme activities, we found that phosphatase and peptidase activities, both phylogenetically broad processes, were generally enhanced in the rhizosphere (Fig. 3a and b). Enhanced phosphatase activities but no change in peptidase activities after the addition of low-molecular-weight organic compounds by a type of artificial root have been reported previously (Renella et al., 2007). Phosphatases and peptidases produced

Table 2
Correlations between microbial processes and abundances of selected marker PLFAs.

PLFA-Markers		N-Mineral.	Nitrific.	Phosph.	Peptidase	Phenox.	Perox.
Gram + bacteria	a15:0	0.05	0	−0.11	0.09	0.26	−0.14
	i16:0	0.47*	0.1	−0.02	0.08	0.27	0
	i17:0	0.32	0.24	0.27	0.31	0.11	0.16
	a17:0	0.11	−0.46+	0.38+	0.43*	−0.57**	−0.5*
Gram − bacteria	cy17:0	0.25	−0.24	0.33	0.32	−0.33	−0.28
	cy18:0	−0.19	0.31	0.34	0.58**	−0.34	−0.2
	18:1ω7	0.17	−0.5+	0.33	0.3	−0.44*	−0.39+
	18:1ω5	0.39+	−0.75***	0.26	0.23	−0.68***	−0.7***
General bacteria	15:0	0.38+	−0.08	−0.19	0.07	0.34	−0.11
	17:0	0.39+	−0.26	0.37+	0.41+	−0.45*	−0.35
Not specified	16:1ω6	0.15	−0.26	0.47*	0.43*	−0.32	−0.31
	i17:1ω8	0.2	0.14	0.67***	0.26	0.12	0.24
Fungi	18:2ω6,9	0.58**	−0.58*	0.17	0.09	−0.5*	−0.54**
	18:1ω9c	0.41+	−0.6*	0.35	0.31	−0.48*	−0.46*
Ubiquitous	16:0	0.52*	−0.29	0.34	0.41+	−0.28	−0.27
	18:0	0.47*	−0.3	0.3	0.42+	−0.22	−0.3

Presented are correlation coefficients. Significance levels are indicated by *** ($p < 0.001$), ** ($p < 0.01$), * ($p < 0.05$), +($p < 0.1$); $n = 6$.

by mycorrhizal fungi probably contribute to enhanced enzyme activities in the rhizosphere, but the pattern of these enzyme activities also reflects the general abundance of bacteria (e.g. the increase in the bulk soil of the girdling treatment).

In contrast, oxidative enzyme activities involved in the degradation of lignin and soil organic matter (phenoloxidase and peroxidase) were lower in the rhizosphere than in bulk soil (Fig. 3c and d). This may be explained by the fact that oxidative enzymes are thought to be produced by slow growing K-strategists, mainly saprotrophic fungi and some specialized groups of bacteria (Buee et al., 2009), which have a competitive disadvantage against mycorrhizal fungi and fast growing copiotrophic bacteria using easily assimilable plant-derived C sources. The reduction in potential oxidative enzyme activities in the rhizosphere compared to bulk soil was not observed in the girdling plots. Our findings hence do not suggest an increase in decomposition of humified SOM by plant exudates ("priming"), as has been reported by others (Bottner et al., 1999; Ekberg et al., 2007; Kuzyakov, 2002). However, although plant exudates have led to reduced activities of oxidative enzymes, they have also accelerated the activities of proteolytic enzymes in our study, and thus may have enhanced the degradation of fresh SOM. Similar results have also been reported recently for cellulytic and other hydrolytic enzymes (Kaiser et al., 2010b). High levels of inorganic N in the girdling plots apparently did not affect the production of oxidative enzymes, contrary to results from a study on the effects of high NO_3^--availability on oxidative enzyme activities in forest soils (Waldrop and Zak, 2006), which reported decreased production of phenoloxidases by white-rot fungi but increased production by bacteria or ascomycetes. The strong negative correlation of oxidative enzyme activities to the abundance of fungal PLFAs seems surprising at first glance (Table 2), but is probably caused by the fact that these biomarkers occur in both mycorrhizal and saprotrophic fungi.

While our results revealed clear rhizosphere effects on microbial processes, microbial community composition in the rhizosphere differed only slightly from bulk soil in controls (ANOSIM $R = 0.083$, $p = 0.19$). The girdling treatment, however, caused a markedly altered community structure at the level of resolution of PLFAs (Fig. 4). Only small rhizosphere effects on microbial community composition in mineral soils have already been reported for grass and tree rhizospheres (Butler et al., 2003; Priha et al., 1999b). We observed no significant differences in the abundance of Gram-positive and Gram-negative bacteria, although the relative abundance of Gram-negative bacteria in the rhizosphere tended to be slightly higher than the relative abundance of Gram-positive bacteria, while in bulk soil the opposite was the case (data not shown). This is consistent with the suggestion that Gram-negative bacteria are more frequent in the rhizosphere, preferably growing on plant labile C, while Gram-positive bacteria may be dominant in bulk soil (Bird et al., in press; Paterson et al., 2007; Treonis et al., 2004). The marked decrease in the abundance of Gram-positive bacteria in rhizosphere soils of girdling plots, which is mainly responsible for the strong shift in community composition, is therefore difficult to explain (Fig. 5c). Since the reduction in labile C supply is unlikely to be the cause, other effects of the girdling treatment on rhizospheric conditions, such as alterations in oxygen supply, pH and redox potential in consequence of reduced root respiration or uptake of nutrients by plants may be possible explanations. Data on fine root biomass (Kaiser et al., 2010b) do not point to increased root mortality or membrane damage of root cells four months after girdling, so these factors can be disregarded. The increase in the abundance of total PLFAs in the bulk soil of the girdling treatment (Fig. 5a), which is also supported by measurements of microbial biomass by the chloroform-fumigation-extraction method (data not shown), may be an effect of the enhanced supply of inorganic N. However, this is likely just a transient effect, since previous results (Kaiser et al., 2010b) showed a reduction of microbial biomass by the girdling treatment throughout most of the seasons.

We found a 28% reduction of the fungal biomarker 18:2ω6,9 in the rhizosphere by girdling as expected (Fig. 5b), but we did not find a reduction of this biomarker in the bulk soil as described in other girdling studies (Högberg et al., 2007), maybe due to an increase in saprotrophic fungi or due to seasonal aspects. The abundance of this fungal biomarker was also not significantly enhanced in the rhizosphere compared to the bulk soil.

Total microbial biomass estimated by total PLFAs was not significantly higher in the rhizosphere (Fig. 5a), although rhizosphere soils are generally considered to be enriched in microbial biomass compared to bulk soil (Cheng and Coleman, 1990; Norton and Firestone, 1991). Results from another study, however, also showed enhanced microbial biomass C only in rhizospheres of spruce, but not of pine and birch (Priha et al., 1999a). Our data may point to a higher microbial activity (activation state) in the rhizosphere at a similar biomass, maybe because of a high availability of easily assimilable carbon and nutrients.

By using a novel approach, i.e by combining the analysis of resource availability, microbial processes and community structure in rhizosphere soil with a tree girdling experiment, we were able to demonstrate that N mineralization rates and hydrolytic enzyme activities were enhanced in the rhizosphere by root exudates, probably an effect of microbial activation by increased resource availability, while activities of oxidative enzymes (involved in the degradation of humified soil organic matter and lignin) were reduced. Thus, we did not find indications of a positive priming effect of root exudates on the degradation of recalcitrant SOM in this study, but rather the opposite effect. Differences in oxidative enzyme activities and nitrification rates between rhizosphere soil and bulk soil suggest considerable differences in the functional microbial community composition, although rhizosphere and bulk soil communities differed only slightly at the level of resolution that we used here (PLFAs). The reduction of root exudations by girdling, on the other hand, led to strong changes of the microbial community in the rhizosphere.

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III. Seasonal variation in functional properties of microbial communities in beech forest soil

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Seasonal variation in functional properties of microbial communities in beech forest soil

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ABSTRACT

Substrate quality and the availability of nutrients are major factors controlling microbial decomposition processes in soils. Seasonal alteration in resource availability, which is driven by plants via belowground C allocation, nutrient uptake and litter fall, also exerts effects on soil microbial community composition. Here we investigate if seasonal and experimentally induced changes in microbial community composition lead to alterations in functional properties of microbial communities and thus microbial processes. Beech forest soils characterized by three distinct microbial communities (winter and summer community, and summer community from a tree girdling plot, in which belowground carbon allocation was interrupted) were incubated with different ^{13}C-labeled substrates with or without inorganic N supply and analyzed for substrate use and various microbial processes. Our results clearly demonstrate that the three investigated microbial communities differed in their functional response to addition of various substrates. The winter communities revealed a higher capacity for degradation of complex C substrates (cellulose, plant cell walls) than the summer communities, indicated by enhanced cellulase activities and reduced mineralization of soil organic matter. In contrast, utilization of labile C sources (glucose) was lower in winter than in summer, demonstrating that summer and winter community were adapted to the availability of different substrates. The saprotrophic community established in girdled plots exhibited a significantly higher utilization of complex C substrates than the more plant root associated community in control plots if additional nitrogen was provided. In this study we were able to demonstrate experimentally that variation in resource availability as well as seasonality in temperate forest soils cause a seasonal variation in functional properties of soil microorganisms, which is due to shifts in community structure and physiological adaptations of microbial communities to altered resource supply.

1. Introduction

Microbial decomposition of soil organic matter (SOM) plays a key role in global C and N cycling (Davidson and Janssens, 2006). It is well known that microbial decomposition processes are controlled, amongst other factors, by substrate quality (e.g. lignin content) and the availability of labile C and nutrients (Chapin et al., 2002; Schmidt et al., 2011). Resource availability influences decomposition processes via effects on microbial physiology, e.g. production of extracellular enzyme activities. Microbial production of extracellular enzymes is stimulated by substrate supply or if available nutrients or C are scarce (Allison and Vitousek, 2005; Hernandez and Hobbie, 2010; Olander and Vitousek, 2000). Enhanced availability of labile C substrates may also increase the decomposition of recalcitrant SOM ('priming effect'), which is ascribed to either microbial activation by the labile C source or enhanced degradation of SOM for the acquisition of limiting nutrients (Blagodatskaya and Kuzyakov, 2008; Fontaine et al., 2011).

Apart from changing the physiology of microbial communities, alterations in resource availability may also influence microbial processes indirectly. As different species of microbes differ in substrate use efficiency and biomass composition and thus demand for C and nutrients (Degens, 1999; Gusewell and Gessner, 2009), changes in resource supply have been shown to induce shifts in

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microbial community composition (Eilers et al., 2010; Fierer et al., 2012; Griffiths et al., 1999; Waldrop et al., 2004). Changes in microbial community composition may in turn strongly affect microbial processes since certain classes of enzymes are produced by specific groups of microorganisms. This is especially true for phylogenetically 'narrow' processes, i.e. processes performed by a relatively small number of specialized species, such as polyphenol degradation, while a higher functional redundancy between different microbial communities may be found for processes performed by a broad array of soil microorganisms, such as C mineralization or protein depolymerization (Balser and Firestone, 2005; Schimel et al., 2005).

While the relation between microbial community composition and function has already been demonstrated in several studies comparing functional properties of microbial communities from different ecosystems (Balser and Firestone, 2005; Brant et al., 2006; Paterson et al., 2011; Strickland et al., 2009; Waldrop and Firestone, 2004), few studies exist investigating the effects of microbial community changes at a single site, e.g. community shifts following increased or decreased plant inputs, on microbial functions (Brant et al., 2006; Paterson et al., 2011; Wickings et al., 2011). It is still not fully understood if and how changes in microbial community composition resulting from seasonal and experimental induced variation in resource availability affect the functional properties of microbial communities, a topic which is especially important in the light of ongoing global change.

In temperate forest ecosystems a seasonal pattern of microbial processes has been observed which is related to a seasonal variation in availability of different substrates, as well as variation in soil temperature and moisture (Kaiser et al., 2011, 2010b). The seasonal variation in resource availability is mainly driven by plants via belowground C exudation and nutrient uptake during the growing season and litter fall in autumn. These seasonal changes in resource availability have also been shown to induce shifts in microbial community structure (Kaiser et al., 2011, 2010b).

This study aimed at elucidating whether changes in the composition of soil microbial communities related to seasonal and experimentally induced variation in resource availability lead to altered functional properties of these microbial communities. We hypothesized (1) that distinct microbial communities that are adapted to different substrates vary in their physiological capacities and in their functional response to addition of various organic substrates and (2) that the functional response of different microbial communities to addition of C substrates is influenced by microbial nutrient limitation.

In order to test these hypotheses we performed an incubation experiment with soils from a beech forest study site (Kaiser et al., 2010b) characterized by three different microbial communities. We chose winter and summer microbial communities as we assumed microbial adaptation to high availability of labile C in summer and to more recalcitrant substrates (litter) in winter. In summer we also collected soils from a tree girdling plot in which belowground carbon allocation had been interrupted which promoted the establishment of a more saprotrophic community. Collecting soils from a single site ensured that soils were comparable in soil properties. We incubated the different soils with a range of labile and complex substrates (glucose, protein, microbial cell walls, cellulose and plant cell walls) and analyzed changes in various microbial processes and pools of labile C and N in response to substrate addition and experimentally enhanced inorganic N supply. By using ^{13}C labeled substrates we were also able to directly monitor microbial substrate utilization and analyze how the various amendments led to changes in the utilization of soil organic matter (i.e. priming).

2. Material and methods

2.1. Origin of 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 a.s.l.). Soils were classified as Dystric Cambisols over flysh (pH in $CaCl_2$ between 4.5 and 5.1) with a mean organic carbon content of 7.45% and nitrogen content of 0.48% in the A horizon. The study site and experimental setup in the field was described in detail previously (Kaiser et al., 2010b), microbial communities were characterized by Kaiser et al. (2010b) and Rasche et al. (2010). 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. Soils for the incubation experiment were collected in February 2008 (winter community) and in June 2008 (summer community and community from girdling plots). 4 subsamples of mineral soil (5 cm depth, A-horizon) were collected from each of 6 replicate plots in winter and summer, respectively, and from 6 replicate girdled plots in summer. Soils from replicate plots were pooled, sieved (5 mm) and stored at 4 °C (winter) and 12 °C (summer) until the start of the incubation experiment. Half of the winter soil was transferred to 12 °C for equilibration 3 days before the incubation.

2.2. Substrates

Five ^{13}C-labeled 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 unlabeled substances, algal protein extract (98 atom % ^{13}C, from Sigma) was applied undiluted.

^{13}C-labeled 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 were described by Keiblinger et al. (2010). Microbial biomass was dried and then resuspended in NaCl-solution. After mechanical 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-labeled plant cell walls were prepared as follows: ^{13}C-labeled wheat roots (IsoLiveBV, U-60402) and unlabeled, dried wheat roots were finely ground and homogenized in a ball mill. The material was then incubated with 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.

2.3. Experimental setup

The respective substrate (1 mg substrate g^{-1} soil of glucose and protein, 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 in order to test the effects of increased N availability on the degradation of C substrates (one of them lignin containing), assuming microbial N limitation in summer.

Incubation of soils (22 g) was performed in a microcosm system with 5 replicates for each substrate and soil (Inselsbacher et al.,

2009). For each soil controls without added substrate were prepared (2 × 5 replicates per soil). The microcosms were loosely closed by 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 walls incubations). Incubation temperature was 12 °C; additionally soils from the winter community were incubated at 4 °C. The process rates of the winter communities relative to their control incubations were not significantly different between 12 °C and 4 °C incubation temperature, thus only the results of 12 °C winter incubations are presented here. At the end of the incubation microcosms were destructively harvested for determination of C and N pools, gross N mineralization, enzyme activities and phospholipid fatty acids.

2.4. Microbial respiration

Microbial respiration rates were measured at 5 time points during the incubation period. At the measurement, 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). 15 ml of headspace gas was 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) were 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:

$$R_{substrate} = APE^{13}C_{resp}/APE^{13}C_{substrate}*100*R_{total}$$

where $APE^{13}C$ means atom % excess ^{13}C in respiration and substrates, respectively, and R_{total} is total respiration.

The change in C mineralization from SOM (including microbial biomass C) induced by addition of organic substrates (='priming effect') was calculated as follows:

$$PE(\%) = ((R_{total} - R_{substrate}) - R_{control})/R_{control}*100$$

where R_{total} is the total respiration from incubations with substrates, $R_{substrate}$ is the respiration from substrate and $R_{control}$ is the respiration from control incubations.

Microbial carbon use efficiency (CUE) was calculated according to the following equation:

$$CUE = {}^{13}C_{mic}/({}^{13}C_{mic} + {}^{13}C_{resp})$$

where $^{13}C_{mic}$ is the amount of substrate ^{13}C in the microbial biomass and $^{13}C_{resp}$ is the cumulative respired substrate ^{13}C.

2.5. Gross N mineralization

Gross N mineralization was assessed using the pool dilution technique (Kaiser et al., 2005; Myrold and Tiedje, 1986). $^{15}NH_4Cl$ was applied to subsamples of fresh soil, incubated for 4 h or 24 h, then extracted with 2M KCl. Ammonium was isolated by the microdiffusion method and ^{15}N values of NH_4^+ were analyzed by an elemental analyzer coupled to an IRMS (DeltaPLUS, Thermo Finnigan). Gross N mineralization rates were calculated according to the following equation:

$$m = (A_t - A_0)/t*(\ln(APE_0/APE_t)/\ln(A_t/A_0))$$

where m is gross mineralization, A_t is the NH_4^+–N pool after time t, A_0 is the initial NH_4^+–N pool, APE (atom % excess) is atom $\%^{15}N - NH_4{}^+{}_{sample}$ − atom $\%^{15}N - NH_4{}^+{}_{natural\ abundance}$.

2.6. C and N pools

Inorganic N was determined from water extracts by chemically suppressed ion-chromatography (Dionex HPAEC on a AS11 column for NO_3^- and Dionex HPCEC on a CS16 column for NH_4^+) (Kaiser et al., 2005). Organic C and total N were analyzed in water extracts by a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu). Microbial biomass C and N was determined by the chloroform fumigation extraction method (Amato and Ladd, 1988). Microbial C and N was estimated from the difference of organic C and total nitrogen measured by a TOC/TN analyzer in KCl extracts of fumigated and unfumigated soils.

2.7. Extracellular enzyme activities

Potential enzyme activities were measured by microplate fluorimetric and photometric assays according to Kaiser et al. (2010b). 4-methylumbelliferyl-β-D-cellobioside, 4-methylumbelliferyl-β-D-N,N′,N″-triacetylchitotrioside and L-leucine-7-amino-4-methyl coumarin, respectively, were used as substrates for the fluorimetric detection of β-1,4-cellobiosidase, endochitinase and leucine amino-peptidase activities. Phenoloxidase and peroxidase activities were measured photometrically after addition of L-3,4-dihydroxyphenylalanine (DOPA).

Actual polysaccharide degradation rates were measured without substrate addition but with the addition of toluene to inhibit microbial uptake of enzymatic products, leading to their accumulation in the soil suspension (Boschker et al., 1995; Kaiser et al., 2010b). Glucose accumulation in the soil solution (analyzed by HPLC) was used for estimation of combined cellulase and amylase activities.

2.8. Phospholipid fatty acids

Phospholipid fatty acids (PLFAs) were extracted by 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). 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 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 indicator of Gram-positive bacteria, the sum of 16:1ω9, 16:1ω7, 18:1ω7, 18:1ω5, cy17:0, cy19:0, cy18:0 as indicator of Gram-negative bacteria, and all these together with 17:0, 17:1ω6, 17:1ω7 as a measure for total bacteria. The quantity of 18:2ω6,9 was used as an indicator of fungal biomass (Kaiser et al., 2010a).

We estimated ^{13}C incorporation into microbial biomass by calculating the weighted average $\delta^{13}C$ value of PLFAs and multiplying it with the amount of microbial biomass C, keeping in mind that $\delta^{13}C$ values may vary between different cell components.

2.9. Statistics

Data were transformed prior to analysis to achieve normality and homogeneity of variances (natural logarithmic transformation

was applied for absolute concentrations and process rates, square root transformation for relative values). Characteristics of the three soils were compared by one-way ANOVA. Multivariate statistics were performed with standardized data. We applied a principal component analysis (PCA) and analysis of similarity (ANOSIM), which is a permutation-based test, to evaluate differences in the functional response of microbial communities to substrate addition. Relative values of microbial processes, C and N pools and microbial groups in percent of control incubations were analyzed for differences from 100% using student's *t*-test. Apart from significance level $p < 0.05$ we also considered $p < 0.1$ as indicating a marginal significance. Differences between microbial communities in incubations of one substrate were assessed using one-way ANOVA and Tukey's post-hoc test, differences between incubations of complex C substrates with and without added inorganic N, respectively, were analyzed using student's *t*-test. Two-way ANOVA was applied for determination of separate effects of substrate and community type on incubations of different substrates. Additionally, three-way ANOVA was applied for determination of the effect of N addition in incubations of complex C substrates. Univariate statistical analyses were performed using Statistica 6.0, multivariate statistics were run with Primer 6.

3. Results

3.1. Characterization of soil and functional microbial communities

Soils collected either in winter or in summer from control plots and from girdled plots were characterized by distinct microbial communities (description of seasonal changes in microbial community composition and effects of tree girdling in Kaiser et al., 2010b) and exhibited differences in availability of C and N and microbial biomass size (Table 1). Nitrate concentrations were significantly higher in soils from the winter harvest than from the summer harvest, while no significant differences could be determined for NH_4^+. Availability of NH_4^+, however, doubled in control incubations of soil collected in winter during the experiment (from 0.21 ± 0.039 to 0.43 ± 0.070 μmol g^{-1} DW) but decreased in summer soil (from 0.19 ± 0.005 to 0.11 ± 0.009 μmol g^{-1} DW) indicating generally higher inorganic N availability in winter than in summer. Total dissolved N concentrations, on the other hand, were highest in soils of girdled plots and lowest in soils collected in winter. Winter soil also exhibited a significantly lower concentration of DOC than the summer soil. Microbial biomass was lowest in girdled plots and had the highest C/N ratio.

Microbial process rates were generally lower in winter than in summer (for detailed description of seasonal variation in microbial processes see Kaiser et al. (2010b)). Actual cellulase/amylase, peptidase and endochitinase activities were lower in girdled plots than in summer control plots, while oxidative enzyme activities were not significantly different (Table S1). If enzyme activities were calculated per unit biomass, however, oxidative enzyme activities were significantly higher in girdled plots than in control plots.

Table 1
Characterization of soils collected in winter, in summer and in summer from girdling plots. Values from soil incubations without substrate addition (winter: 4 °C, summer 12 °C). Mean values ($n = 5$). Significant differences (by Tukey's post-hoc test; $p < 0.05$) are indicated by different letters.

	Winter	Summer	Summer-girdling
NH_4^+ (μmol g^{-1} DW)	0.21^a	0.19^a	0.15^a
NO_3^- (μmol g^{-1} DW)	1.73^b	0.65^a	0.78^{ab}
Total dissolved N (μmol g^{-1} DW)†	2.5^a	3.3^b	4.6^c
Dissolved organic C (μmol g^{-1} DW)	8.4^a	13.6^b	14.4^b
Microbial C (μmol g^{-1} DW)	31.7^{ab}	37.0^b	24.2^a
Microbial N (μmol g^{-1} DW)	5.1^{ab}	6.9^b	3.4^a
Microbial C/N ratio	6.2^{ab}	5.5^a	7.2^b
Net N mineralization (nmol NH_4–N g^{-1} DW h^{-1})	-6.1^a	-1.8^a	3.0^a

†Measured in KCl-extracts.

3.2. Changes in C and N pools by substrate addition

Addition of glucose significantly increased microbial C and C/N ratio in soils collected in winter, in summer and from girdled plots (Table S2). Incubations of microbial cell walls, cellulose and plant cell walls exhibited reduced microbial biomass (both C and N) in soil collected in summer from control plots while the first two substrates revealed the opposite effect in soil from girdled plots. Microbial C was not affected by N addition in either of the communities. Addition of cellulose and plant cell walls significantly increased microbial N in winter (148 and 156% of control incubations, respectively), which translated into a marked decrease in C/N ratio of microbial biomass (63 and 66% of controls, respectively; $p < 0.001$).

NH_4^+ concentrations significantly increased with protein addition in soils collected in winter, summer and from girdled plots (Table S2). Glucose and cellulose incubations of winter soil exhibited markedly reduced NH_4^+ concentrations compared to control incubations (43 and 33%, respectively; $p < 0.001$), while in soil from girdled plots NH_4^+ availability was enhanced by the addition of cellulose.

3.3. Changes in microbial processes by substrate addition/microbial substrate utilization

A principal component analysis of 7 microbial process rates (C mineralization, SOM mineralization, substrate ^{13}C in respiration, actual cellulase/amylase, cellobiosidase, peptidase, and chitinase activities) relative to unamended controls revealed that the functional response of microbial communities to substrate addition depended on the type of added substrate, glucose and protein incubations being set apart from incubations of microbial cell walls and complex C substrates (Fig. 1). While the three different microbial communities responded similarly to the addition of microbial cell walls and protein, clear functional differences between microbial communities were observed for the C substrates. Winter community significantly differed from the summer community in the response to addition of both labile C (glucose) and complex C substrates (cellulose, plant cell walls; ANOSIM $p < 0.05$). Significant differences between communities from summer control plots and girdled plots were found in incubations of complex C substrates ($p < 0.05$). Enhanced inorganic N availability clearly influenced the functional response of the community from control plots to addition of complex C substrates ($p < 0.05$), while a significant effect of N on the community from girdled plots was found in incubations of cellulose only. The first PCA axis mainly separating the glucose and protein incubations from the other substrates was negatively correlated to concentrations of microbial C and microbial C/N ratio (relative to control incubations) and positively correlated to NH_4^+, NO_3^- and total dissolved N. The second PCA axis separating the winter community as well as the community from girdled plots from the summer community showed negative correlations to concentrations of microbial C and N, NH_4^+ and also the abundance of fungi (relative to control incubations). Factor loadings on PCA 1 were most negative for respiration, APE^{13}C in respiration and cellulase/amylase activity, while cellobiosidase, endochitinase and peptidase activities showed highest loadings on PCA 2.

Respiration was more than doubled by the addition of labile C in incubations of all microbial communities, the increase being

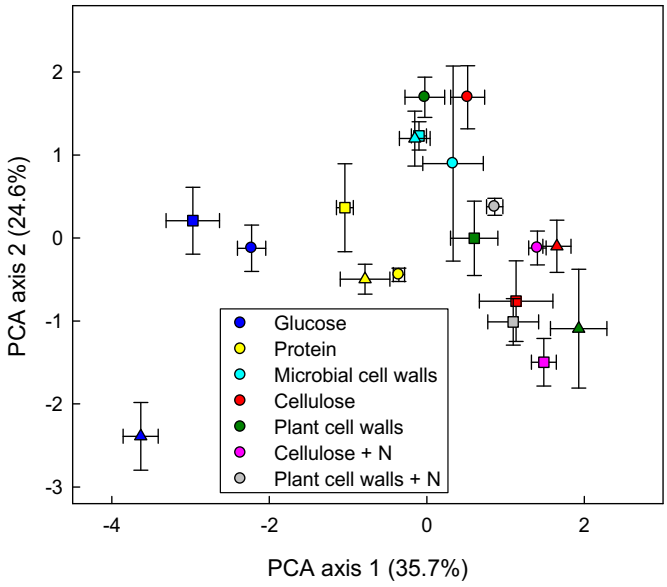

Fig. 1. Results of a principal component analysis from 7 microbial process rates (C mineralization, priming effect of SOM mineralization, substrate ^{13}C in respiration, actual cellulase/amylase, cellobiosidase, peptidase, and chitinase activities) relative to control incubations without substrate addition in soils collected in winter (triangles), in summer (circles) and in summer from girdling plots (squares), which were incubated with five organic substrates with or without inorganic N supply. Values are means ± SE ($n = 4$).

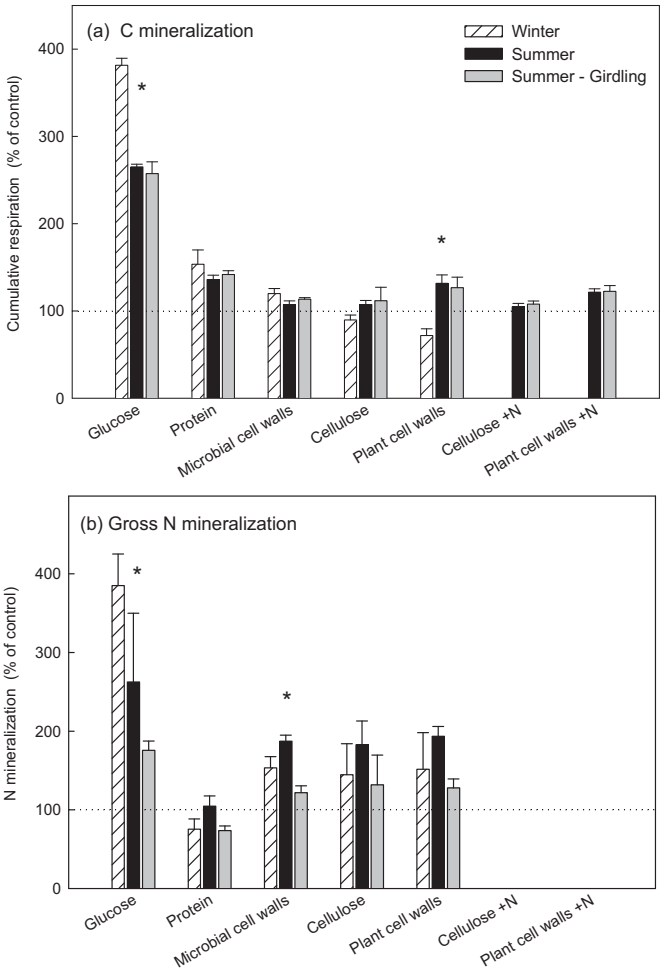

Fig. 2. Changes in (a) cumulative C mineralization and (b) gross N mineralization rates (relative to unamended controls) in response to addition of different organic substrates and inorganic N in soils collected in winter (hatched bars), in summer (black bars) and in summer from girdling plots (gray bars). Values are means ± SE. (a) $n = 4$, (b) $n = 5$. Significant differences between communities in incubations of a certain substrate (determined by ANOVA, $p < 0.05$) are indicated by asterisks (*). Values of unamended controls are given in Table S1. Incubation time was 2 days for glucose and protein incubations and 6 days for incubations of microbial cell walls, cellulose and plant cell walls.

significantly higher in winter than in summer (Fig. 2a). Addition of a complex C substrate, plant cell walls, however, stimulated respiration in summer only, while a significant decline in cumulative respiration was observed in winter ($p < 0.05$). Increased inorganic N availability did not affect cumulative respiration in soils from either control or girdled plots. The different response in respiration of winter and summer communities mainly reflected differences in mineralization of soil organic matter rather than respiration of the added substrates. In winter a marked priming effect of SOM decomposition by glucose was observed ($p < 0.001$) (Fig. 3a), while addition of plant cell walls reduced mineralization of SOM ($p < 0.05$). In summer, however, a positive priming effect by plant cell walls was found ($p < 0.05$). ANOVA results hence revealed a highly significant community × substrate interaction for both cumulative respiration and priming of SOM decomposition but no main effect of community (Table 2).

Differences in carbon use efficiency between the different communities may also contribute to the observed pattern of respiration. Winter community exhibited lower efficiency of glucose utilization than summer community but higher efficiency in the use of plant cell walls (Fig. 3b). As indicated by substrate ^{13}C concentrations in respiration and microbial biomass (Table 3), glucose, protein and microbial cell walls were generally more intensively used by microbes than the polymeric C substrates. Uptake of added glucose was higher in summer than in winter, while the opposite was found for protein. The microbial community in soils from girdled plots tended to use the complex C substrates more intensively than the summer control community, a tendency considerably enforced by the addition of inorganic N. Enhanced inorganic N availability strongly increased the concentration of ^{13}C in respiration in both soils from control and girdled plots ($p < 0.001$). A comparably strong effect of N addition on substrate ^{13}C in microbial biomass was only found in cellulose incubations of soils from girdled plots.

ANOVA results for gross N mineralization revealed significant effects of community, substrate and community × substrate interaction (Table 2). Glucose addition significantly stimulated N mineralization, with the highest increase being found in winter (Fig. 2b). Interestingly, protein exhibited no or a negative effect, while microbial cell walls, cellulose and plant cell walls increased gross N mineralization, especially in incubations of the summer community.

Significant effects of community, substrate as well as community × substrate interaction were also observed for all of the measured extracellular enzyme activities (Table 2).

Actual cellulase/amylase activities were strongly stimulated by glucose, the enzymatic product, as well as by microbial cell walls (Fig. 4a). Winter community significantly differed from summer community in the response to complex C substrates. While in winter actual cellulase activity was stimulated by cellulose and plant cell walls amendments ($p < 0.001$), no effect was found in summer. The pattern of cellobiosidase activity, indicating potential cellulolytic activity, differed considerably from actual cellulolytic activity, with an increase in plant cell wall incubations of the summer community ($p < 0.05$) but a decrease in cellulose incubations of the community from girdled plots ($p < 0.01$) (Fig. 4b).

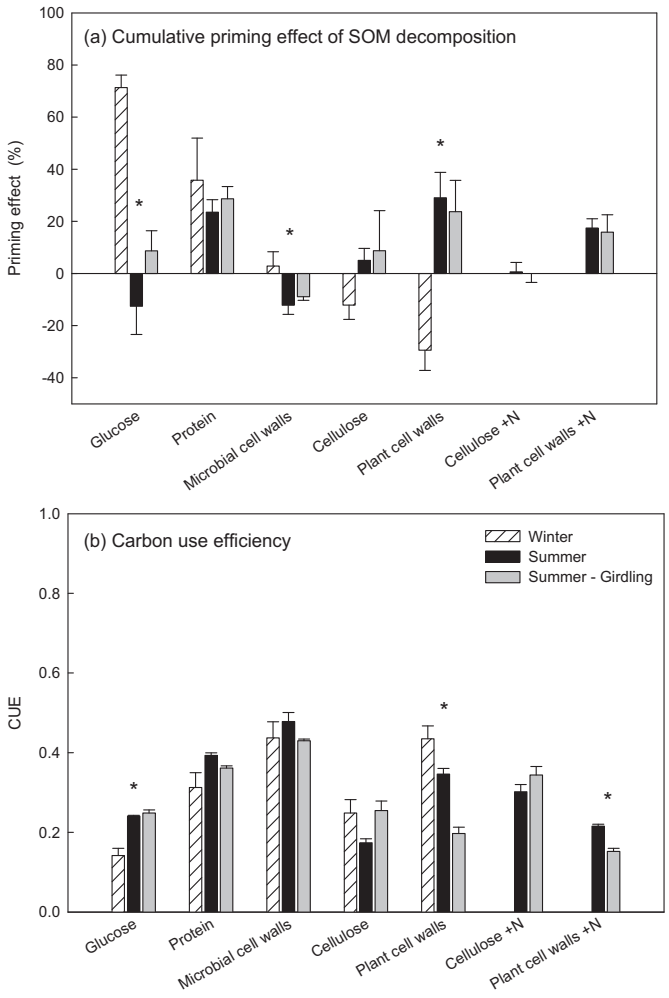

Fig. 3. (a) Cumulative priming effect of SOM respiration induced by addition of different organic substrates and inorganic N and (b) microbial carbon use efficiency calculated from respired substrate ^{13}C and substrate ^{13}C in microbial biomass (see 2.4) in soils collected in winter (hatched bars), in summer (black bars) and in summer from girdling plots (gray bars). Values are means ± SE ($n = 4$). Significant differences between communities in incubations of a certain substrate (determined by ANOVA or *t*-test, $p < 0.05$) are indicated by asterisks (*).

Both actual cellulase/amylase and potential cellobiosidase activities, however, showed the same trends in the response of communities to inorganic N addition, separating summer (control) community from the community from girdled plots. While in soils from control plots cellulolytic activity decreased in plant cell wall incubations with high N availability compared to plant cell wall incubations without added N ($p < 0.05$), the community from girdled plots showed a significantly positive response to inorganic N addition in cellulose incubations ($p < 0.05$), which was also depicted by a significant community × N interaction for cellulolytic enzymes (Table 2).

Differences between summer and winter communities in response to complex C substrates were also revealed by peptidase activities (Fig. 4c). In summer peptidase activity was significantly enhanced by addition of cellulose ($p < 0.05$) and plant cell walls ($p < 0.001$) while in winter the opposite effect was observed. The community from girdled plots tended to exhibit a lower response to these substrates than the community from control plots. Addition of inorganic N significantly reduced peptidase activity ($p < 0.001$). Endochitinase, responsible for the depolymerization of N-rich microbial cell walls, showed a similar, although less significant response to N addition and similar differences between communities in plant cell wall incubations (Fig. 4d).

Oxidative enzyme activities were significantly enhanced by the addition of plant cell walls in both soils from control and girdled plots, as well as by microbial cell walls in soil collected in winter (Fig. 4e and f). Stimulation of phenoloxidase activity by plant cell walls was higher in soil from girdled plots than from control plots ($p < 0.05$). Inorganic N addition exerted little effect on oxidative enzyme activities, only a marginally significant decrease in peroxidase in soil from control plots was found ($p < 0.1$).

3.4. Changes in abundances of microbial groups by substrate addition

Substrate addition generally had a stronger influence on fungal abundance than on bacterial abundance (Table S3). We observed a marginally significant increase in fungal abundance after glucose addition ($p < 0.1$) and a strong decrease after addition of cellulose and plant cell walls in soil collected in summer from control plots (79 and 49% of control incubations, respectively; $p < 0.05$), while in soil from girdled plots a slightly positive response to cellulose was observed. Enhanced inorganic N supply increased the abundance of fungi in soils from both control and girdled plots ($p < 0.05$). A positive effect of organic N, however, was observed in soil from

Table 2
ANOVA results showing effects of community type, substrate and inorganic N addition on alterations of various microbial processes. n.d. means 'not determined'. Values in bold indicate $p < 0.05$.

	dF	C mineralization		Gross N mineralization		Priming effect		C use efficiency		Cellulase/Amylase	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Community	2	0.14	0.8684	6.57	**0.0027**	1.00	0.3747	2.66	0.0842	15.68	**0.0000**
Substrate	4	193.50	**0.0000**	15.23	**0.0000**	9.22	**0.0000**	69.39	**0.0000**	147.82	**0.0000**
Community × Substrate	8	12.77	**0.0000**	1.75	0.1066	11.27	**0.0000**	12.84	**0.0000**	18.44	**0.0000**
+N	1	0.52	0.4789	n.d.	n.d.	1.84	0.1881	0.91	0.3515	1.37	0.2497
Community × N	1	0.05	0.8181	n.d.	n.d.	0.00	0.9812	1.14	0.2977	6.76	**0.0140**
	dF[a]	Cellobiosidase		Peptidase		Endochitinase		Phenoloxidase		Peroxidase	
		F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Community	2	6.44	**0.0029**	10.68	**0.0001**	11.08	**0.0001**	6.81	**0.0136**	5.85	**0.0214**
Substrate	4	3.55	**0.0115**	2.89	**0.0294**	6.88	**0.0001**	6.67	**0.0013**	9.91	**0.0001**
Community × Substrate	8	4.70	**0.0002**	4.73	**0.0002**	3.80	**0.0012**	4.96	**0.0062**	4.59	**0.0088**
+N	1	0.64	0.4307	101.99	**0.0000**	13.05	**0.0010**	0.05	0.8202	1.03	0.3259
Community × N	1	9.65	**0.0039**	0.00	0.9582	0.01	0.9190	0.59	0.4553	0.10	0.7595

[a] dF Oxidative enzyme activities: 1, 3, 3, 1, 1 (summer only).

Table 3
Microbial utilization of ^{13}C-labeled substrates by the winter, summer and summer-girdling community indicated by concentrations of substrate ^{13}C in respiration and microbial biomass (estimated from PLFAs). Mean values; $n = 4$ (respiration), $n = 3$ (microbial biomass). Significant differences (by Tukey's post-hoc test; $p < 0.05$) are indicated by different letters. n.d. means 'not determined'.

	Substrate ^{13}C in respiration (atom % excess ^{13}C)			Substrate ^{13}C in microbial biomass (atom % excess ^{13}C)		
	W	S	G	W	S	G
Glucose	10.4^{a}	12.6^{b}	10.9^{ab}	0.84^{a}	1.14^{b}	1.13^{ab}
Protein	11.4	8.9	8.9	1.40^{b}	1.00^{a}	1.12^{a}
Microbial cell walls	2.7^{a}	3.4^{b}	3.7^{b}	1.91	1.51	2.09
Cellulose	0.31	0.31	0.41	0.06^{ab}	0.04^{a}	0.09^{b}
Cellulose + N	n.d.	0.59^{a}	1.07^{b}	n.d.	0.14^{a}	0.42^{b}
Plant cell walls	0.29^{a}	0.36^{ab}	0.43^{b}	0.11	0.11	0.08
Plant cell walls + N	n.d.	0.65^{a}	1.00^{b}	n.d.	0.11^{a}	0.16^{b}

control plots only. The response of fungal abundance to substrate addition was negatively correlated to changes in peroxidase activity in incubations of the summer community ($p < 0.05$), while in incubations of the community from girdled plots a positive correlation of fungal abundance with peroxidase activity ($p < 0.05$) and negative correlations with peptidase and endochitinase activities ($p < 0.05$) were observed. In contrast to fungal abundance, the total bacterial abundance was not significantly affected by substrate addition (Table S3).

4. Discussion

Previous studies have demonstrated a seasonal variation in microbial decomposition processes in temperate forest soils which was related to a seasonal shift in availability of substrates and a seasonal variation in soil temperature and moisture (Kaiser et al., 2011, 2010b). In these studies the reduction of belowground C allocation by tree girdling strongly affected microbial processes, as also reported by others (Ekberg et al., 2007; Högberg et al., 2001; Subke et al., 2004). Seasonal and experimentally induced alterations in resource availability and abiotic factors, however, have also been shown to induce changes in microbial community composition (Fierer et al., 2012; Kaiser et al., 2010b; Lauber et al., 2008; Yarwood et al., 2009). Here we demonstrate experimentally that microbial community changes due to alterations in resource availability result in functional differences between microbial communities implying that the distinct microbial communities differ in their physiological capacities. In agreement with our expectations, the functional response of microbial communities to substrate addition depended on the type of added substrate (Fig. 1, Table 2), presumably since extracellular enzyme activities as well as C and N mineralization rates vary with resource supply and stoichiometry (Allison and Vitousek, 2005; Geisseler and Horwath, 2009; Hernandez and Hobbie, 2010). In addition, we also observed a significant influence of community type on relative process rates (Fig. 1, Table 2) underlining functional differences between microbial communities, especially in the decomposition of complex C substrates. This is in line with other studies also reporting functional differences between distinct microbial communities (Brant et al., 2006; Fierer et al., 2012; Paterson et al., 2011).

When interpreting these results, it has to be kept in mind, however, that the soils collected at different seasons and from girdled plots differed in both microbial community composition and availability of labile C and N, which implies that effects of resource availability and effects of community structure or physiological adaptations of microbes may combine here. We experimentally enhanced inorganic N availability in summer soils, which facilitates separation of these effects.

Soil collected in winter was characterized by lower availability of DOC but higher availability of inorganic N than summer soil (Table 1). The relatively low C/N ratio of microbial biomass in summer soil was probably due to the fact that soil was sampled at the beginning of summer, prior to the sharp decrease in microbial biomass N usually observed at this site in July (Kaiser et al., 2011). Regardless of differences in resource availability our results revealed considerable functional differences between summer and winter communities in response to addition of C substrates, reflecting microbial adaptation to availability of different types of C sources in summer and winter. The winter community responded to the addition of complex C substrates with significantly enhanced actual cellulase/amylase activity (Fig. 4a) and reduced mineralization of soil organic matter (Fig. 3a). Both suggest adaptation of the winter community to degradation of complex C substrates, such as plant litter, which is also reflected by the high carbon use efficiency for plant cell walls in this community (Fig. 3b). In summer, on the contrary, actual cellulase/amylase activity was not influenced by the addition of plant cell walls and was decreased by the additional enhancement of inorganic N availability. This may indicate that the effects on cellulolytic enzyme activities (and SOM mineralization) observed in plant cell wall incubations of the winter community were not due to higher N availability in winter but reflected a functional property of the winter community. The marked increase in microbial biomass N and decrease in biomass C/N ratio in response to addition of polymeric C substrates in winter (Table S2) is consistent with our previous results showing a phase of enhanced microbial N uptake and N storage in microbial biomass during winter months (Kaiser et al., 2011). Glucose addition caused a considerably stronger increase in C mineralization in winter than in summer (Fig. 2a). This was mainly due to a strong priming effect of glucose on SOM mineralization in winter (Fig. 3a), which may reflect a depletion of labile carbon in winter that may have been overcome by the pulse of labile C. Enhanced turnover of microbial C pools after microbial activation may also contribute to this effect ('apparent priming effect' (Blagodatskaya and Kuzyakov, 2008)). The summer community, on the other hand, seemed to be more adapted to utilization of labile C sources (which are known to be supplied to microbes during summer by plant roots (Dennis et al., 2010; Jones et al., 2009)), as exemplified by higher uptake rates of glucose compared to the winter community (Table 3) and higher carbon use efficiency for glucose (Fig. 3b). Activity of peptidase (and chitinase; Fig. 4c/d) was increased by complex C substrates in summer (contrasting to negative effects in winter), while inorganic N addition reduced peptidase and chitinase activities, as reported from other studies (Allison and Vitousek, 2005; Olander and Vitousek, 2000). The increase in N-acquiring enzymes by the addition of cellulose and plant cell walls in summer may indicate microbial N limitation during the plant growing season and can be related to a positive priming effect of SOM decomposition (Fig. 3a), suggesting 'microbial N mining' (Fontaine et al., 2011).

In a previous study it was demonstrated that tree girdling significantly reduced the abundance of mycorrhizal fungi. Increased input of dead fine root biomass resulted in enhanced saprotrophic activity and high biomass turnover two years after girdling (Kaiser et al., 2010b). This is also indicated by oxidative enzyme activities and high levels of dissolved (organic) N, as well as high gross N mineralization in soils from girdled plots in our study (Tables 1 and S3). Differences in the response to substrate addition between microbial communities from girdled and control plots are thus likely to reflect the different functional properties of a purely saprotrophic community and a microbial community shaped by plant roots.

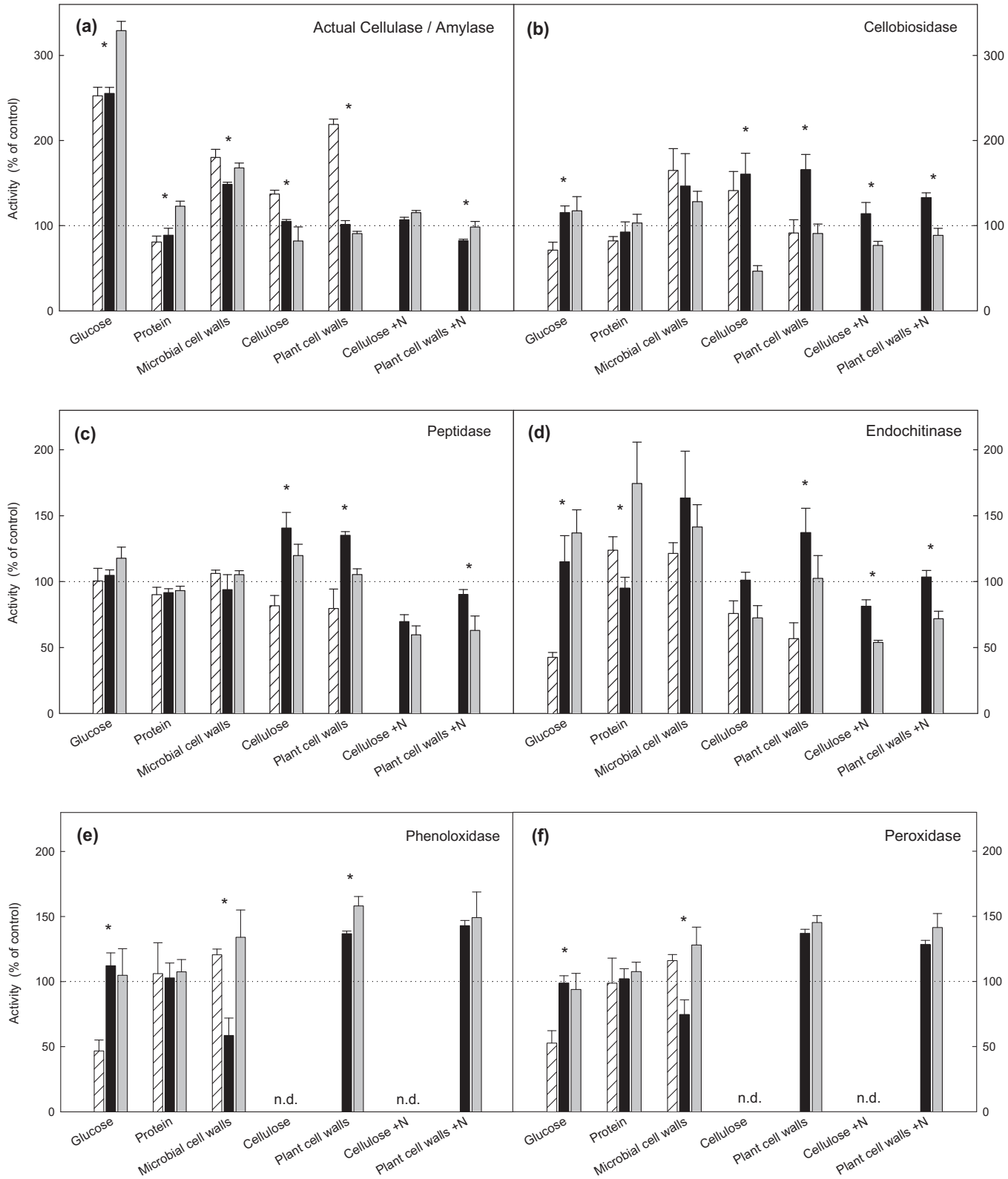

Fig. 4. Changes in extracellular enzyme activities (relative to unamended controls) in response to addition of different organic substrates and inorganic N in soils collected in winter (hatched bars), in summer (black bars) and in summer from girdling plots (gray bars). Values are means ± SE ($n = 5$). Significant differences between communities in incubations of a certain substrate (determined by ANOVA or *t*-test, $p < 0.05$) are indicated by asterisks (*). Values of unamended controls are given in Table S1. Incubation time was 2 days for glucose and protein incubations and 6 days for incubations of microbial cell walls, cellulose and plant cell walls.

Oxidative enzyme activities were enhanced in both soils from control and girdled plots by the addition of plant cell walls, but the increase in phenoloxidase activity was significantly higher in the soil from girdled plots. Enhanced oxidative enzyme activities in plant cell wall incubations may arise from the degradation of the lignin-containing substrate or from enhanced degradation of SOM (priming) (Fig. 3a). Increased inorganic N concentration showed little effect on oxidative enzyme activities, in contrast to other studies reporting negative (Carreiro et al., 2000; Sinsabaugh et al., 2002) but also positive effects (Saiya-Cork et al., 2002) of N fertilization or additions. However, as revealed by ^{13}C concentrations in respiration and microbial biomass (Table 3), microbial utilization of complex C substrates was markedly enhanced by inorganic N addition and was significantly higher in soils from girdled plots than from control plots, which suggests a higher capacity of the saprotrophic community in girdled plots for degradation of these substrates, reflecting microbial adaptation to high availability of dead root biomass in girdled plots.

Interestingly, cellulolytic enzyme activities responded differently to N addition in soils from girdled and control plots. In soil from girdled plots N addition increased cellulolytic enzyme activity in cellulose incubations (Fig. 4a and b), consistent with results from previous studies (Allison and Vitousek, 2005; Geisseler and Horwath, 2009; Saiya-Cork et al., 2002; Sinsabaugh et al., 2002), whereas activity of these enzymes was decreased by inorganic N in soil from control plots. N limitation of the microbial community in girdled plots is unlikely to be the cause since lower peptidase and chitinase activities in these incubations indicate higher N availability in soils from girdled plots. Differences in the response of cellulolytic enzyme activities to N addition might hence suggest that different species of microbes were responsible for the degradation of complex C substrates in control plots and girdled plots, as indicated by their different response to N. It also suggests that N addition caused a community shift which was different in soils from control and girdled plots. Relating enzyme activities to changes in the abundances of different microbial groups, especially the abundance of fungi (Table S3) may help to clarify this. Counter to the current paradigm, the abundance of fungi was strongly decreased by the addition of complex C substrates in soil from control plots (pointing to cellulose-degrading bacteria outcompeting those fungal taxa adapted to labile C sources), while the opposite trend was observed in incubations of soil from girdled plots. But in both soils, abundance of fungi was enhanced by inorganic N addition. The fact that, unlike soil from girdled plots, soil from control plots exhibited a different response of cellulolytic enzyme activities and the abundance of fungi to N addition might indicate that fungi may be less involved in degradation of polymeric C substrates in control plots and that instead other members of the microbial community play an important role in cellulolytic activity. Differences in physiological capacities of fungi in control and girdling plots, respectively, are also indicated by positive correlations between relative peroxidase activity and fungal abundance in soil from girdled plots, but negative correlations in soil from control plots.

In summary our results revealed that microbial communities in soils collected at different seasons and from experimentally altered plots clearly differed in their response to substrate addition. The observed pattern in microbial processes reflects distinct physiological capacities of winter and summer communities. The winter community revealed a higher capacity for degradation of complex C substrates (cellulose, plant cell walls) but lower utilization of labile C sources (glucose) than the summer community. The saprotrophic community established in girdled plots exhibited a significantly higher utilization of complex C substrates compared to the community from control plots if additional nitrogen was provided.

It can thus be concluded that the plant-induced variation in resource availability in temperate forest soils leads to a seasonal variation in functional properties of soil microorganisms, resulting from seasonal changes in microbial community structure and physiological adaptations of microorganisms.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2013.01.025.

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Supplement

Table S1. Microbial process rates in unamended control incubations of soils collected in winter, in summer and in summer from girdling plots incubated for 2 or 6 days. Mean values (n = 5). Differences between microbial communities (by Tukey's post-hoc test, $p<0.05$) are indicated by different letters (2 days: lower case letters, 6 days: capital letters). MUF, methylumbelliferyl; AMC, aminomethylcoumarin; DOPA, L-3,4-dihydroxyphenylalanin.

	Winter		Summer		Summer - Girdling	
	2 days	6 days	2 days	6 days	2 days	6 days
Cumulative C mineralization (µmol CO_2 g^{-1} DW)	5.0 ^{a}	21.6 ^{A}	4.9 ^{a}	17.9 ^{A}	4.6 ^{a}	18.1 ^{A}
Gross N mineralization (nmol NH_4-N g^{-1} DW h^{-1})	7.9 ^{a}	9.7 ^{A}	19.7 ^{b}	12.1 ^{AB}	27.3 ^{b}	23.0 ^{B}
Cellulase / Amylase (µg gluc g^{-1} DW h^{-1})	6.5 ^{a}	8.0 ^{A}	13.2 ^{b}	16.7 ^{C}	6.9 ^{a}	12.6 ^{B}
Cellobiosidase (nmol MUF g^{-1} DW h^{-1})	71.1 ^{a}	50.0 ^{A}	133.9 ^{b}	59.3 ^{AB}	106.9 ^{b}	97.9 ^{B}
Peptidase (nmol AMC g^{-1} DW h^{-1})	96.7 ^{ab}	94.5 ^{A}	113.2 ^{b}	82.0 ^{A}	92.0 ^{a}	81.1 ^{A}
Endochitinase (nmol MUF g^{-1} DW h^{-1})	20.2 ^{ab}	35.4 ^{A}	33.3 ^{b}	23.1 ^{A}	13.6 ^{a}	21.9 ^{A}
Phenoloxidase (µmol DOPA g^{-1} DW h^{-1})	0.54 ^{a}	1.03 ^{A}	1.06 ^{b}	0.91 ^{A}	1.07 ^{b}	0.92 ^{A}
Peroxidase (µmol DOPA g^{-1} DW h^{-1})	1.12 ^{a}	1.62 ^{A}	1.64 ^{b}	1.37 ^{A}	1.78 ^{b}	1.53 ^{A}

Table S2. Changes (in % of unamended controls) in C and N pools in soils collected in winter, in summer and in summer from girdled plots in response to addition of different substrates and inorganic N. Mean values (n = 5). Values in bold indicate significant differences from control incubations (by student's t-test; $p<0.05$). n.d. means 'not determined'.

Relative values (% of control)	Microbial C			Microbial N			Microbial C/N ratio		
	W	S	G	W	S	G	W	S	G
Glucose	**149**	**118**	**154**	100	**86**	**135**	**150**	**135**	**115**
Protein	96	109	**118**	93	110	**137**	101	97	**86**
Microbial cell walls	79	**89**	**110**	80	**86**	**117**	101	**103**	95
Celllulose	93	**86**	**121**	**148**	**90**	**129**	**63**	**96**	**94**
Cellulose + N	n.d.	**85**	**109**	n.d.	106	n.d.	n.d.	95	n.d.
Plant cell walls	103	**87**	90	**156**	**82**	98	**66**	**107**	99
Plant cell walls + N	n.d.	**85**	100	n.d.	98	n.d.	n.d.	99	n.d.

	NH_4^+			NO_3^-			Total dissolved N			Dissolved organic C		
	W	S	G	W	S	G	W	S	G	W	S	G
Glucose	**43**	96	112	99	97	**63**	**80**	**88**	**75**	103	106	106
Protein	**295**	**173**	**165**	204	75	100	**142**	**131**	98	**138**	**135**	**133**
Microbial cell walls	89	**138**	120	102	**92**	104	91	**137**	107	107	**127**	107
Celllulose	**33**	158	**388**	123	**87**	**117**	**83**	**118**	**110**	123	**114**	**110**
Cellulose + N	n.d.	>1000	>1000	n.d.	154	88	n.d.	>1000	>1000	n.d.	**114**	**124**
Plant cell walls	119	121	**281**	**142**	**78**	**137**	88	89	**83**	103	90	**83**
Plant cell walls + N	n.d.	>1000	>1000	n.d.	129	120	n.d.	>1000	>1000	n.d.	**106**	**111**

Table S3. Changes (in % of unamended controls) in abundances of microbial groups in soils collected in winter, in summer and in summer from girdled plots in response to addition of different substrates and inorganic N. Mean values (n = 3). Values in bold indicate significant differences from control incubations (by student's t-test; $p<0.05$; values in grey $p<0.1$). n.d. means 'not determined'.

(Abundance in % of control)	Fungi			Total bacteria			Gram- bacteria			Gram+ bacteria		
	W	S	G	W	S	G	W	S	G	W	S	G
Glucose	96	133	118	100	111	135	95	**124**	121	110	92	159
Protein	101	**133**	109	94	100	94	92	105	101	97	92	80
Microbial cell walls	**84**	84	144	92	144	134	92	134	138	91	162	127
Celllulose	102	**79**	129	90	102	152	91	108	**142**	88	93	170
Cellulose + N	n.d.	102	**203**	n.d.	115	108	n.d.	96	106	n.d.	147	101
Plant cell walls	91	**49**	118	87	92	90	91	**83**	**106**	81	108	66
Plant cell walls + N	n.d.	**62**	**156**	n.d.	97	123	n.d.	85	108	n.d.	117	145

IV. Fungal and bacterial utilization of complex organic substrates is differentially influenced by 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. This study aimed at elucidating whether differences in substrate utilization between distinct microbial communities are due to activities of certain microbial groups. We performed an 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-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 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. At high N availability fungi dominated degradation of polymeric C substrates, while at low N availability bacteria played an important role in cellulose degradation, which indicates that bacteria and fungi may be functionally redundant in degradation of complex C substrates and that their competitive abilities are strongly influenced by nutrient availability.

Introduction

Soil microorganisms processing the decomposition and mineralization of soil organic matter (SOM) play a crucial role in the terrestrial C and N cycle (Chapin *et al.*, 2002; Schmidt *et al.*, 2011). Due to the high diversity of soil microorganisms it has long been assumed that there is a functional redundancy between 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). This hypothesis, however, has been questioned 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 elucidate 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 years for monitoring substrate utilization of microbial groups (at a low phylogenetic resolution) is the analysis of carbon isotope ratios of microbial phospholipids fatty acids (PLFAs) combined with stable isotope labeling. In several studies it was observed that labile plant derived C was predominantly metabolized by Gram-negative bacteria and fungi, while Gram-positive bacteria seemed to be involved in the degradation of SOM (Treonis *et al.*, 2004; Bird *et al.*, 2011). Furthermore, degradation of complex and N poor substrates was mainly ascribed to fungi, while bacteria rather 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 allowing 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).

Such a strict distribution of functional roles of microbial groups, however, has been questioned since bacteria and fungi have also been reported to compete for the same substrates indicating a functional redundancy (De Boer *et al.*, 2005; Strickland & Rousk, 2010). Added labile C substrates were found to be equally utilized by a broad range of

community members (Waldrop & Firestone, 2004; Paterson *et al.*, 2011). There is also evidence of competition between bacteria and fungi for complex substrates (Rousk *et al.*, 2008; Strickland & Rousk, 2010), which is in line with observations that certain bacterial species have the physiological capacities for degradation of polymeric C substrates and even lignified substrates (Perestelo *et al.*, 1996; Vargas-Garcia *et al.*, 2007). The question of functional differences or functional redundancy between different microbial groups is hence still not completely solved, especially in the context of different nutrient requirements of microbial groups and 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 activities of certain microbial groups. We hypothesized (1) that distinct microbial communities differ in utilization of organic substrates and in partitioning of substrate derived C within the microbial community and (2) that fungal and bacterial substrate utilization is differentially influenced by enhanced inorganic N availability.

We performed an experiment with beech forest soils characterized by three microbial communities (winter and summer community, as well as community from a tree girdling plot where the bark had been removed from trees to disrupt belowground C allocation), which we incubated with different ^{13}C-labeled 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 of 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 a.s.l.). Soil was classified as Dystric Cambisol over flysh (pH in $CaCl_2$ betweeen 4.5 and 5.1) with a mean organic carbon content of 7.45% and nitrogen content of 0.48% in the A horizon. Soil was collected in February 2008 (winter community) and in 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. Experimental setup in the field, as well as microbial processes and microbial communities were described in detail by Kaiser *et al.* (2010); Rasche *et al.* (2010); Kaiser *et al.* (2011). Soil was stored at 4°C (winter) and 12°C (summer) 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 were described in Koranda *et al.* (2013), microbial community composition (determined from phospholipid fatty acids) is given in Table 1. Although relative abundances of major microbial groups did not differ significantly between the soils, a PCA calculated from all PLFA abundances revealed distinct microbial community composition of soils collected in winter, 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, algal protein extract (98 atom % ^{13}C, 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 were described by Keiblinger *et al.* (2010). Microbial biomass was dried and then resuspended in NaCl-solution. After mechanical 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, U-60402) and unlabelled, dried wheat roots were mixed and finely ground and homogenized in a ball mill. The material was then incubated with 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.

Experimental setup

The respective substrate (1 mg substrate g^{-1} soil of glucose and protein (corresponding to ~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 in order to test the effects of increased N availability on the degradation of C substrates (one of them lignin containing), assuming microbial N limitation in summer.

Incubation of soils (22 g) was performed in a microcosm system with 4 replicates for each substrate and soil (Inselsbacher et al., 2009). For each soil controls without added substrate were prepared (2 x 4 replicates per soil). Microcosms were loosely closed by 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 walls incubations). Incubation times of the experiment were chosen with the aim to include the peak of $^{13}CO_2$ release following the addition of substrates. 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 substrate-C in the winter community did not differ significantly between both 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). 15 ml 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 minutes. Concentration and carbon isotope ratio of CO_2 (relative to VPD) was determined via a GasBench II interfaced to continous-flow isotope ratio mass spectrometry (IRMS; Delta

V Advantage,Thermo Fisher, Germany). Respiration from substrate was calculated according to the following equation:

$R_{substrate} = APE^{13}C_{resp} / APE^{13}C_{substrate} * 100 * R_{total}$

Where $APE^{13}C$ means atom % excess ^{13}C in respiration and substrates, respectively, and R_{total} is total respiration.

Phospholipid fatty acids

Phospholipid fatty acids (PLFAs) in soils were extracted by 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 in order 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 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 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 indicator of Gram-negative bacteria and the fatty acids 15:0 and 17:0 as indicator for bacteria in general (Zelles, 1997; Leckie, 2005). The PLFA 16:1ω5 has also been reported as a marker for arbuscomyrrhizal fungi (Olsson, 1999). However, as in beech forest soils ectomycorrhiza is the dominant form of mycorrhiza and no other mycorrhizal type was detected in our soil samples, 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 was used as a marker for protozoa (Leckie, 2005).

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 program Primer 6 for ANOSIM, Statistica 6.0 for all other analyses.

Results

Microbial substrate utilization

Cumulative respiration from substrate (integrated over 33 hours for labile substrates and over 5.2 days for complex substrates) was highest for glucose, followed by microbial cell walls (Table 2). For 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 could be observed, most likely because also complex substrates contained a small amount of monomers (Fig. S1). Respiration of complex substrates (microbial cell walls, cellulose and plant cell walls) increased from the third day until the end of the incubation period (except for cellulose incubations without inorganic N).

Microbial communities in soils collected in winter or summer significantly differed in the respiration of labile substrates (Table 2). While the summer community more intensively respired C from glucose compared to the winter community, a higher respiration of added protein by the winter community was observed. 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.

At the end of the incubation period the amount of substrate derived C in PLFAs was highest in incubations of microbial cell walls, followed by glucose incubations (Table 2). The summer community had incorporated 3-fold as much C from glucose as the winter community. Substrate incorporation in 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 than the community in control plots at high N availability.

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 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 from control plots at both low and high N availability (except for substrate incorporation from plant cell walls; data not shown).

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 added substrate (Fig. 1a; ANOSIM $p = 0.01$, $R = 0.836$). 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 to incubations without N. Factor loadings on PCA-axis 1 were highest for fungal marker PLFAs and two marker PLFAs for Gram-negative bacteria (Fig. 1b). Bacterial markers had 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 of different microbial groups (in percent of total incorporated substrate C; Fig. 2). Gram-negative bacteria generally incorporated the highest proportion of total substrate derived C (at low N availability), with especially high values being found in incubations of microbial cell walls (~47%), followed by cellulose (33 - 41%) and glucose incubations (~36%). Gram-positive bacteria incorporated the highest proportion of substrate C in cellulose incubations (between 20 and 27%). Fungi most actively metabolized C from glucose (20-23 % of total incorporated C) and plant cell walls (17-20 %). In winter fungi incorporated a significantly higher proportion of protein than in summer (19% compared to 15.3%, respectively). Highest substrate incorporation in the marker for protozoa was observed in incubations of the two N-rich substrates, microbial cell walls and protein.

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.

2). While the proportion of substrate C in fungal markers was significantly enhanced by N addition to cellulose and plant cell wall incubations, the proportion 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, while absolute bacterial substrate utilization was not significantly changed (data not shown). 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 (data not shown). ^{13}C incorporation of Gram-negative bacteria also tended to decline at high N availability in incubations of plant cell walls.

Contrasting 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 S1).

^{13}C concentrations of specific phospholipid fatty acids

^{13}C from glucose was detected in nearly all the analyzed PLFAs (Fig. 3a), highest $\delta^{13}C$-values being measured in the fungal marker 18:2ω6 in soil from girdled plots. It has to be kept in mind, that differences in $\delta\ ^{13}C$-value of a certain fatty acid between soils (and also differences in $\delta\ ^{13}C$ -values between different fatty acids) do not necessarily mean differences in (absolute) ^{13}C-incorporation, as they may be due to different concentrations of fatty acids.

In protein incubations (Fig. 3b), substrate ^{13}C was also recovered in most of the fatty acids, similar to the pattern in glucose incubations. Highest $\delta\ ^{13}C$ -values were measured in soil collected in winter in the protozoan marker 20:4ω6, as well as in the fatty acid 16:0 (the precursor molecule for other phospholipid fatty acids (Abraham et al., 1998)), and in the fatty acid i17:1ω8.

The pattern of ^{13}C-concentrations in incubations microbial cell walls (Fig. 3c), another N-containing substrate, differed considerably from the pattern observed in protein incubations. Only four PLFAs were highly labeled: 20:4ω6, 16:0, and two markers for Gram-negative bacteria, 16:1ω7 and 18:1ω7.

δ ^{13}C-values in incubations of complex C substrates (cellulose and plant cell walls, Fig. 3d and 3e) were much lower than for the first three substrates. In cellulose incubations, the highest label was detected in PLFA 16:1ω5 (Gram-negative bacteria) in soil from girdled plots, while in plant cell wall incubations highest δ ^{13}C -values were measured in the fungal markers 18:2ω6 and 18:3ω3. ^{13}C-concentrations in these fungal markers were markedly enhanced by increased inorganic N availability in cellulose incubations (in both soils from control and girdled plots) and in plant cell wall incubations in soils from girdled plots, while the high ^{13}C-incorporation from cellulose in PLFA 16:1ω5 nearly dropped to zero at high N availability. According to changes in δ ^{13}C -values of marker PLFAs for Gram-positive bacteria by inorganic N addition, two different types of Gram-positive marker PLFAs (probably characterizing different genera or families) could be distinguished: While δ ^{13}C -values of the PLFAs i15:0 and i17:0 were decreased by N addition in both cellulose and plant cell wall incubations, δ ^{13}C -values of i16:0 and a17:0 were increased.

Discussion

In this study we investigated differences in 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 (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 (Fig.1, 2). 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). Functional differences between summer and winter communities in utilization of another labile substrate, protein, however, seemed to be linked to fungal activity, as indicated by higher fungal substrate incorporation in winter soil.

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 three years after girdling. In cellulose incubations, as well as cellulose and plant cell walls incubations with inorganic N additions, microbial communities from girdled and control plots also exhibited a differing pattern of substrate incorporation of microbial groups (Fig. 1, 2), 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 already 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. Our results revealed, however, 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 bacteria played an important role in cellulose degradation, especially in soil from girdled plots, while fungi played a minor role (Fig. 2, 3). The capacity of different bacterial taxa for degradation of cellulose has already been reported in other studies (De Boer *et al.*, 2005; Vargas-Garcia *et al.*, 2007; Goldfarb *et al.*, 2011). 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. Fungi more intensively utilized plant cell walls than cellulose (Fig. 2, 3), which is probably due to the lignin content of plant cell walls, as well as N contained in cell wall proteins (0.83% N in plant cell walls).

If inorganic N was added to cellulose and plant cell walls, fungal decomposition activity of polymeric C substrates strongly increased (Fig. 2, 3). This positive effect of N addition on fungal activity is remarkable, since 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). Stimulation of fungal growth or activity by enhanced N availability, however, has also been found in other studies (Rousk & Baath, 2007; Boberg *et al.*, 2008; Meidute *et al.*, 2008; Fontaine *et al.*, 2011). The often observed

fungal dominance in the decomposition of N-poor plant litter hence seems to be to a great extent 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). The fact that N addition had a stronger effect on fungal activity in soil from girdled plots than from control plots (despite higher availability of dissolved N in soil from girdled plots compared to control plots, data not shown) points to the occurrence of different fungal species with different N demands in girdled and control plots (probably purely saprotrophic species in soil from girdled plots compared to both saprotrophic and initially plant-root associated species in soil from control plots).

Bacterial incorporation of polymeric substrates, on the other hand, tended to be reduced by N addition (Fig. 2, 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). Marker PLFAs for Gram-positive bacteria showed divergent responses to N fertilization, which suggests a shift within Gram-positive bacterial species by enhanced N availability.

Our results on microbial utilization of polymeric C substrates corroborated findings by others (Rousk et al., 2008), who observed that fungi and bacteria may compete for the same complex substrates, indicating functional redundancy between microbial groups with respect to decomposition processes. Competitive abilities of microbial groups apparently strongly depend on N availability, fungi outcompeting bacteria at high N availability as main decomposers of complex C-rich substrate. The fact that this was accompanied by substantially increased decomposition rates indicates that fungi are probably more efficient decomposers of complex C substrates compared to bacteria, but stronger limited by N in these forest soils.

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 activities of a certain microbial group, but reflected differences in substrate utilization by a large part of the microbial community. This contradicts our first hypothesis with respect to labile C substrates. 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, confirming hypothesis (2). At high N availability saprotrophic fungi clearly dominated decomposition of polymeric C substrates, while at low N availability bacteria played an important role in decomposition of cellulose. Our results hence revealed that bacteria and fungi may be functionally redundant with respect to degradation of polymeric C substrates and that their competitive abilities are strongly influenced by changes in nutrient availability, with far-reaching consequences for decomposition rates.

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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)

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). Mean values; n = 4 (respiration) or n = 3 (PLFAs). Significant differences (Tukey's post-hoc test; $p<0.05$) are indicated by different letters. n.d. 'not determined'.

	Respired substrate derived C ($\mu g\ g^{-1}$ DW)			Substrate derived C in PLFAs ($\mu g\ g^{-1}$ DW)		
	W	S	G	W	S	G
Glucose	90.5 [a]	126.5 [b]	91.0 [a]	3.33 [a]	10.05 [b]	7.47 [b]
Protein	10.2 [a]	9.3 [b]	8.7 [b]	1.94	1.59	1.21
Microbial cell walls	44.8	42.5	48.9	15.16	15.42	13.42
Cellulose	5.2	5.2	6.7	0.55	0.37	0.75
Cellulose + N	n.d.	9.2 [a]	16.9 [b]	n.d.	1.42 [a]	3.10 [b]
Plant cell walls	3.0 [a]	5.2 [b]	5.9 [b]	0.87	0.72	0.40
Plant cell walls + N	n.d.	8.4 [a]	12.8 [b]	n.d.	0.64	0.93

Legends to figures

Figure 1

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).

Figure 2

Relative substrate C incorporation (in % of total incorporated substrate C) of microbial groups, estimated from ^{13}C incorporation of marker PLFAs. 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.

Figure 3

^{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).

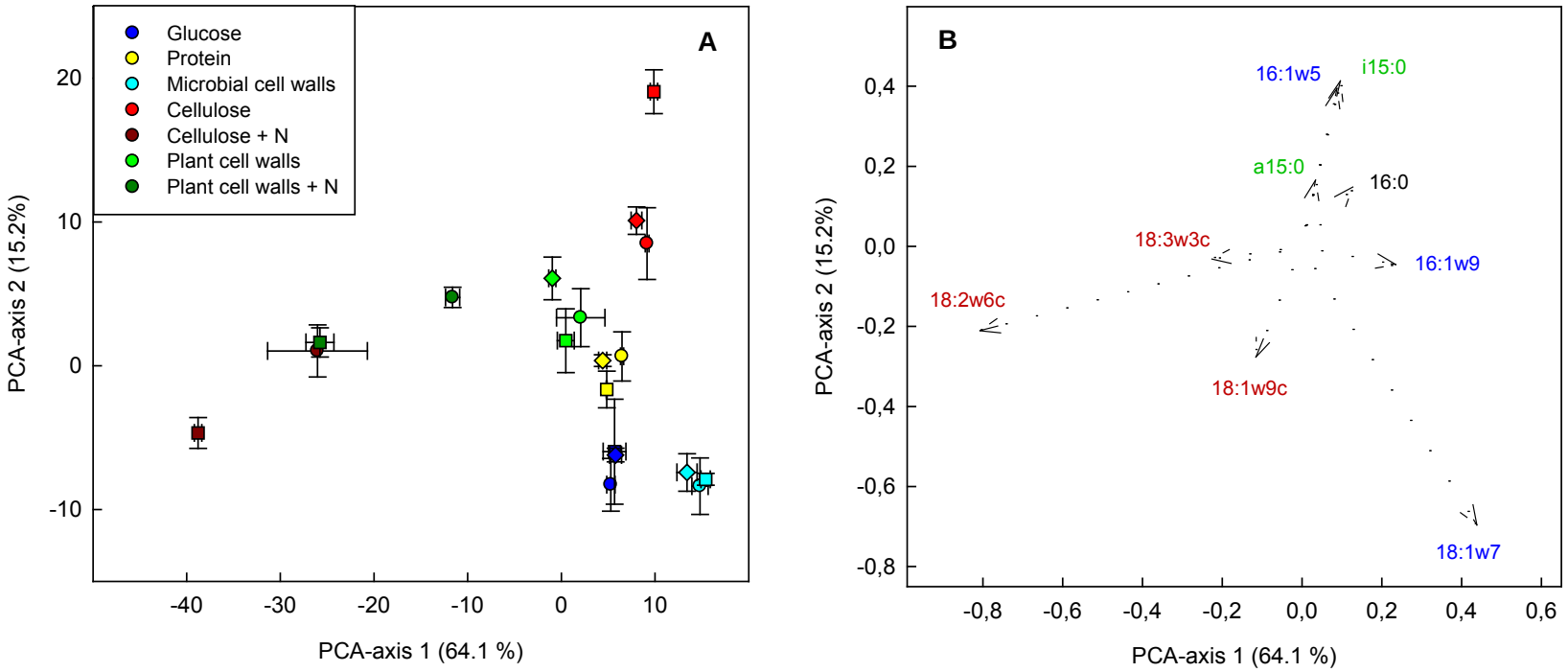
A
Glucose
Protein
Microbial cell walls
Cellulose
Cellulose + N
Plant cell walls
Plant cell walls + N
PCA-axis 2 (15.2%)
PCA-axis 1 (64.1 %)
20
10
0
-10
-40
-30
-20
-10
0
10
B
16:1w5
i15:0
a15:0
16:0
18:3w3c
16:1w9
18:2w6c
18:1w9c
18:1w7
PCA-axis 2 (15.2%)
PCA-axis 1 (64.1 %)
0,4
0,2
0,0
-0,2
-0,4
-0,6
-0,8
-0,8
-0,6
-0,4
-0,2
0,0
0,2
0,4
0,6

Fig. 1

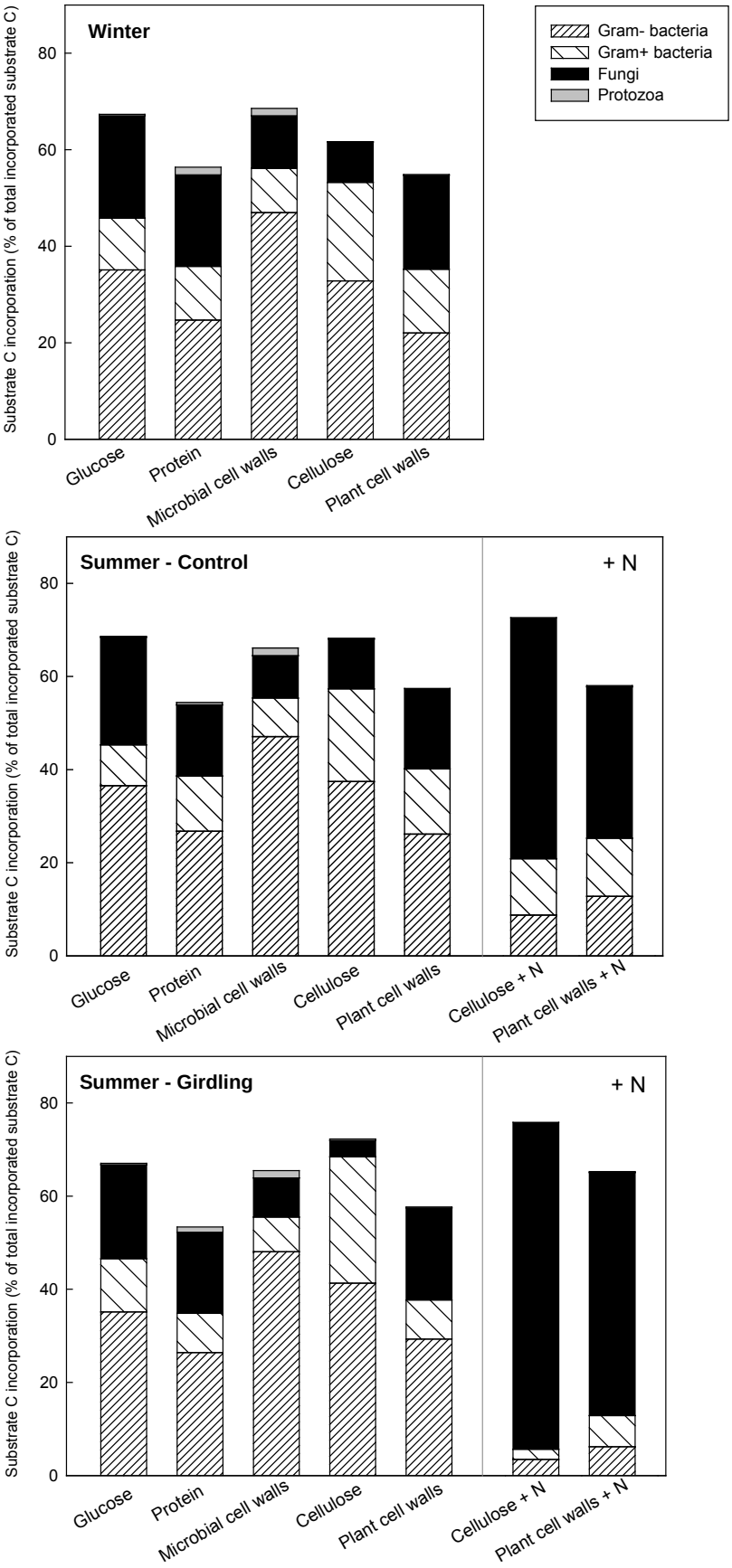
Winter
Gram- bacteria
Gram+ bacteria
Fungi
Protozoa
Substrate C incorporation (% of total incorporated substrate C)
0
20
40
60
80
Glucose
Protein
Microbial cell walls
Cellulose
Plant cell walls
Summer - Control
+ N
Cellulose + N
Plant cell walls + N
Summer - Girdling

Fig. 2

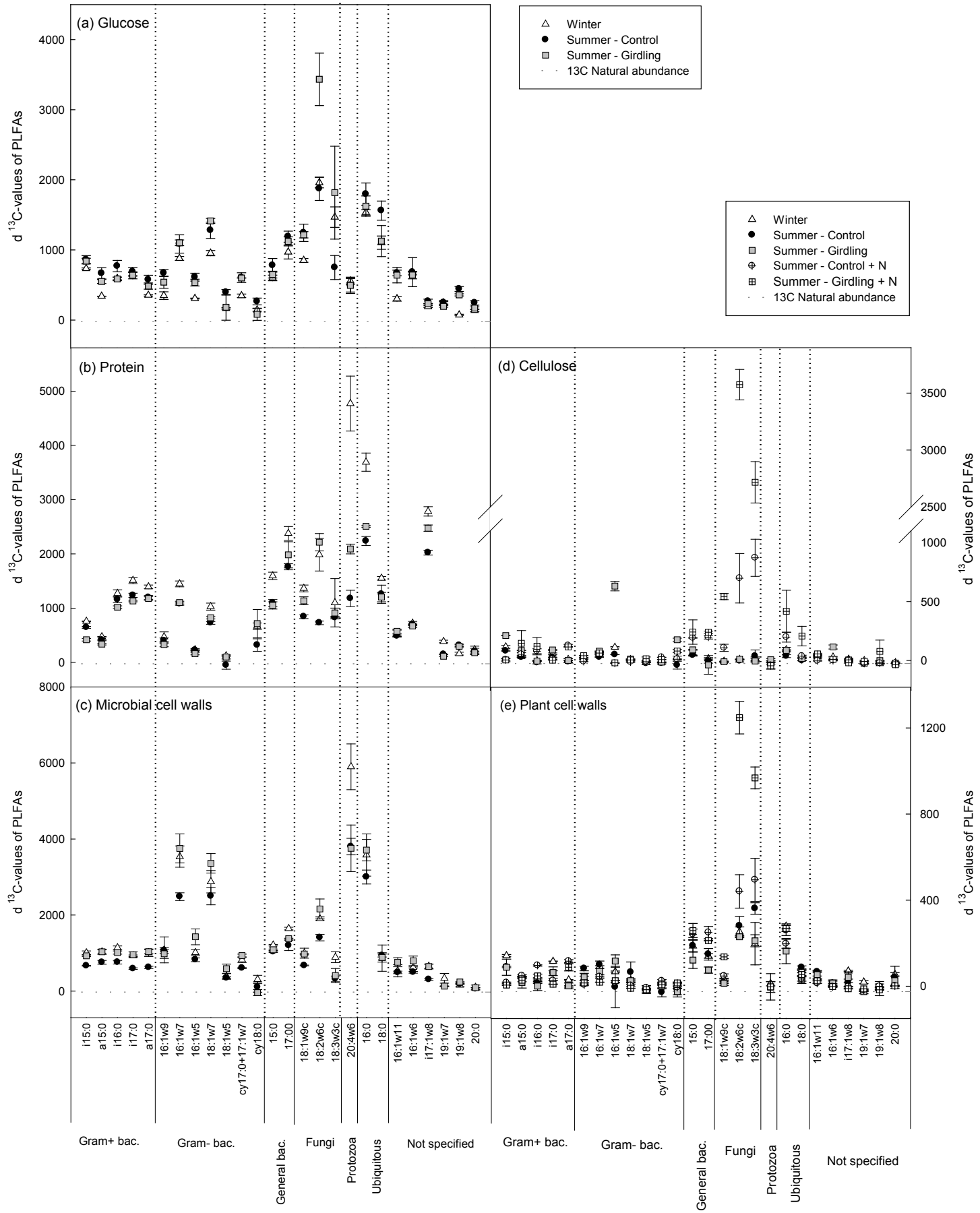
(a) Glucose
(b) Protein
(c) Microbial cell walls
(d) Cellulose
(e) Plant cell walls
Winter
Summer - Control
Summer - Girdling
Summer - Control + N
Summer - Girdling + N
13C Natural abundance
d 13C-values of PLFAs
i15:0
a15:0
i16:0
i17:0
a17:0
16:1w9
16:1w7
16:1w5
18:1w7
18:1w5
cy17:0+17:1w7
cy18:0
15:0
17:00
18:1w9c
18:2w6c
18:3w3c
20:4w6
16:0
18:0
16:1w11
16:1w6
i17:1w8
19:1w7
19:1w8
20:0
Gram+ bac.
Gram- bac.
General bac.
Fungi
Protozoa
Ubiquitous
Not specified

Fig. 3

Table S1. 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. Values are means of three, SE in brackets.

Winter	Total bacteria		Gram+ bacteria		Gram- bacteria		Fungi		Protozoa	
Glucose	51.5	(2.0)	17.8	(2.2)	32.2	(0.5)	12.5	(0.4)	0.30	(0.04)
Protein	51.0	(1.9)	18.6	(1.4)	30.7	(0.5)	14.3	(1.1)	0.37	(0.04)
Microbial cell walls	50.4	(0.1)	17.8	(0.7)	30.9	(0.7)	12.9	(0.2)	0.36	(0.05)
Cellulose	49.7	(1.0)	17.2	(1.2)	30.5	(0.9)	14.4	(0.7)	0.35	(0.03)
Plant cell walls	49.0	(2.5)	16.3	(2.6)	31.2	(1.2)	15.4	(1.2)	0.43	(0.05)
Summer - Control										
Glucose	48.6	(1.8)	14.3	(2.7)	32.7	(1.0)	15.0	(1.1)	0.33	(0.11)
Protein	49.4	(1.4)	16.1	(2.7)	31.4	(1.4)	15.3	(1.3)	0.35	(0.02)
Microbial cell walls	50.8	(1.8)	19.0	(2.6)	30.1	(0.9)	12.6	(0.5)	0.51	(0.06)
Cellulose	46.9	(1.8)	13.9	(4.0)	31.3	(2.4)	15.2	(1.8)	0.48	(0.04)
Cellulose +N	49.4	(1.2)	21.5	(0.3)	26.1	(0.8)	14.4	(0.6)	0.32	(0.02)
Plant cell walls	52.0	(0.3)	20.8	(0.4)	29.1	(0.3)	12.4	(0.4)	0.15	(0.15)
Plant cell walls +N	45.9	(2.7)	18.1	(1.9)	26.1	(0.8)	13.6	(0.3)	0.17	(0.17)
Summer - Girdling										
Glucose	49.2	(2.2)	19.7	(5.1)	27.9	(2.9)	10.1	(1.7)	0.50	(0.10)
Protein	45.5	(0.3)	13.5	(0.1)	30.1	(0.3)	11.7	(0.5)	0.46	(0.01)
Microbial cell walls	47.3	(0.1)	16.5	(0.7)	29.2	(0.7)	11.3	(0.6)	0.67	(0.11)
Cellulose	49.1	(1.9)	19.8	(2.7)	27.7	(0.9)	11.1	(0.8)	0.57	(0.04)
Cellulose +N	42.6	(1.4)	14.4	(2.9)	25.3	(0.2)	14.6	(0.2)	0.40	(0.04)
Plant cell walls	44.0	(1.0)	11.5	(1.5)	30.7	(0.5)	13.3	(0.3)	0.68	(0.02)
Plant cell walls +N	46.2	(2.6)	19.5	(4.0)	25.0	(1.6)	12.3	(1.3)	0.53	(0.01)

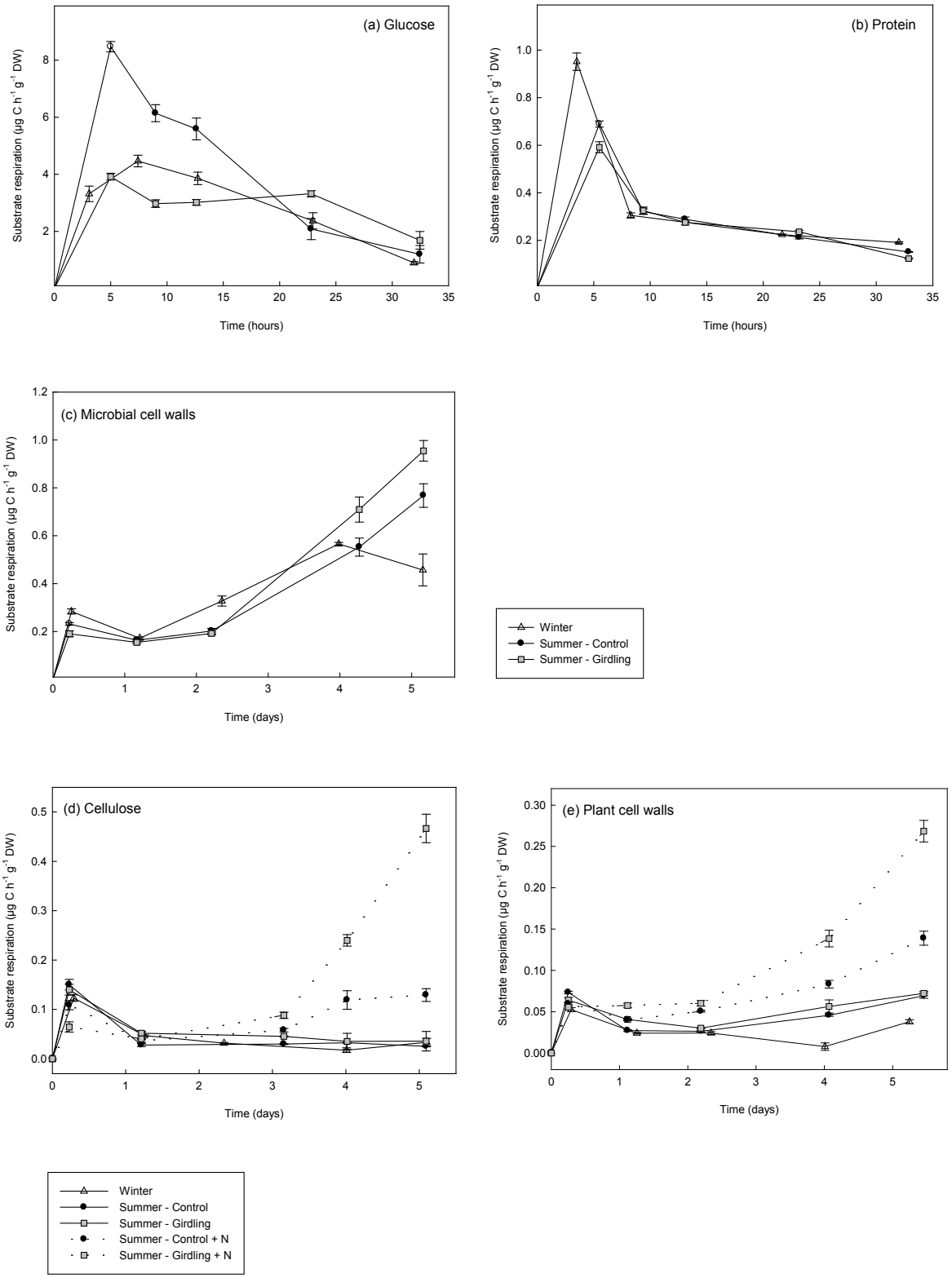

Figure S1. Substrate respiration in soils collected in winter (triangles), in summer from control plots (circles) and from girdled plots (squares), incubated with five organic substrates with or without inorganic N addition. Values are means ± SE (n = 3

V. SUMMARY / ZUSAMMENFASSUNG

This thesis aimed at elucidating the relationship between resource availability, microbial community composition and microbial decomposition processes of soil organic matter. We performed a field experiment in a temperate beech forest (combining a two-year-seasonal experiment with a tree girdling experiment) and a soil incubation experiment with various organic substrates.

Our results revealed that alterations in substrate availability driven by plants via litter fall, belowground carbon allocation and nutrient uptake exert strong effects on microbial decomposition processes and community composition. Seasonal alterations in resource availability, as well as variation in abiotic factors, lead to a clear seasonal pattern in extracellular enzyme activities and microbial biomass dynamics, which was also related to shifts in microbial community composition. Reduction of belowground C allocation by tree girdling resulted in a markedly altered microbial community structure (especially a reduction in the abundance of mycorrhizal fungi), with strong consequences on microbial process rates.

Effects of plant-induced changes in resource availability were also obvious if microbial processes in the rhizosphere of beech roots were compared to bulk soil processes. High availability of labile C in the rhizosphere and resulting microbial activation lead to high gross N mineralization rates and hydrolytic enzyme activities. Oxidative enzyme activities (which are involved in the degradation of SOM), however, were lower in the rhizosphere than in bulk soil, which was probably an effect of the dominance of mycorrhizal fungi over saprotrophic fungi in the rhizosphere.

By an incubation experiment performed with soils from the beech forest study site, we were able to demonstrate that seasonal changes in resource availability and the resulting shifts in microbial community structure lead to a seasonal variation in functional properties of microbial communities, reflecting adaptations of microbial communities to availability of different substrates. Results of this experiment, in which we incubated soils collected at different seasons and from girdled plots with different organic substrates and inorganic N supply, revealed that the winter community exhibited a higher capacity for degradation of complex substrates (cellulose, plant cell walls), while the summer community more intensively used labile C substrates (glucose). The saprotrophic community in girdled plots exhibited a higher capacity for degradation of complex C substrates than the community in control plots, if additional N was provided.

The analysis of substrate -^{13}C incorporation into different microbial groups (by PLFA-analysis) showed that substrate C derived from labile C sources was equally distributed

within the microbial communities. The active microbial community involved in degradation of complex C substrates, however, differed between girdled and control plots and was strongly influenced by inorganic N addition. At high N availability fungi were the main decomposers of polymeric C substrates, while at low N availability bacteria played an important role in cellulose degradation, which suggests a certain functional redundancy between fungi and bacteria for degradation of the same substrates, but differences in nutrient requirements.

In conclusion, this thesis demonstrated a clear relationship between resource availability and microbial functions. Availability of different substrates types, as well as supply of labile C and N, strongly influenced microbial processes and also affected microbial community structure and functional properties of microbial communities. Effects of microbial community composition on microbial functions were most apparent for degradation of complex C substrates, such as plant cell walls, and recalcitrant SOM. This suggests that only specialized microbial taxa have the physiological capacities for degradation of these substrates while the capacity for utilization of labile substrates, such as protein, is more widespread among soil microorganisms.

Ziel dieser Arbeit war die Erforschung der Zusammenhänge zwischen Ressourcenverfügbarkeit, mikrobieller Gemeinschaftsstruktur und mikrobiellen Abbauprozessen von organischer Substanz in Waldböden. Zu diesem Zweck führten wir in einem Buchenwald in Niederösterreich über 2 Jahre einen Freilandversuch durch, u.a. auch mit Baumberingelungen. Weiters umfasste unser Projekt einen Boden-Inkubationsversuch mit unterschiedlichen organischen Substraten.

Unsere Ergebnisse zeigten, dass Änderungen in der Ressourcenverfügbarkeit, die von Pflanzen durch Laubfall, Nährstoffaufnahme und Kohlenstoff-Transfer in den Boden verursacht werden, starke Auswirkungen auf mikrobielle Abbauprozesse und die Zusammensetzung der mikrobiellen Gemeinschaften im Boden haben. Infolge von jahreszeitlichen Änderungen der Substratverfügbarkeit und Schwankungen in abiotischen Faktoren stellten wir saisonale Muster in extrazellulären Enzymaktivitäten und mikrobiellem Wachstum fest, die auch mit Änderungen in der mikrobiellen Gemeinschaftsstruktur korrelierten. Baum-Beringelung und die damit verbundene Unterbrechung des C-Transfers in den Boden hatte starke Auswirkung auf die Zusammensetzung der mikrobiellen Gemeinschaften (insbesondere bewirkte es eine

Reduktion der Mykorrhiza-Pilze), was sich auch deutlich in geänderten mikrobiellen Prozessraten zeigte.

Auswirkungen von durch Pflanzen bedingten Änderungen in der Ressourcenverfügbarkeit auf mikrobielle Prozesse beobachteten wir auch in Untersuchungen der Rhizosphäre. Hohe Verfügbarkeit von labilem C in der Rhizosphäre und die daraus resultierende hohe mikrobielle Aktivität äußerten sich in erhöhten Brutto N-Mineralisierungsraten und hydrolytischen Enzymaktivitäten. Hingegen waren oxidative Enzymaktivitäten, die wesentlich am Abbau von organischer Substanz beteiligt sind, in der Rhizosphäre niedriger als im übrigen Boden, was vermutlich auf die Dominanz von Mykorrhizapilzen über saprophytische Pilze zurückzuführen ist.

In einem Inkubationsversuch von Buchenwald-Böden zeigte sich, dass jahreszeitliche Schwankungen in der Ressourcenverfügbarkeit und dadurch bedingte Änderungen in der mikrobiellen Gemeinschaftsstruktur auch Änderungen in den funktionellen Eigenschaften der mikrobiellen Gemeinschaften bewirken, infolge von Anpassung an unterschiedliche Substrate. Die Ergebnisse dieses Versuches, bei dem Böden zu unterschiedlichen Jahreszeiten bzw. von Baumberingelungs-Flächen gesammelt und mit verschiedenen organischen Substraten und anorganischem Stickstoff inkubiert wurden, verdeutlichten, dass die Winter-Gemeinschaften eine höhere Fähigkeit zum Abbau von komplexen C-Substraten (Zellulose, pflanzlichen Zellwänden) hatten als die Sommer-Gemeinschaften, während die Verwendung von labilen C-Substraten (Glukose) im Winter niedriger war als im Sommer. Die mikrobiellen Gemeinschaften in Baumberingelungs-Flächen hatten eine höhere Kapazität zum Abbau von komplexen C-Substraten als die Gemeinschaften in den Kontroll-Flächen bei Vorhandensein von zusätzlichem anorganischem N.

Die Analyse der Substrat-Nutzung durch unterschiedliche mikrobielle Gruppen mittels ^{13}C-PLFA-Methode zeigte, dass C aus labilen C-Substraten innerhalb der mikrobiellen Gemeinschaften gleichmäßig verteilt war. Die mikrobiellen Gruppen, die am Abbau von komplexen C-Substraten beteiligt waren, unterschieden sich jedoch zwischen Beringelungs- und Kontrollflächen und wurden stark durch Änderungen in der N-Verfügbarkeit beeinflusst. Bei hoher N-Verfügbarkeit wurden komplexe C-Substrate hauptsächlich durch Pilze abgebaut, während bei niedriger N-Verfügbarkeit Bakterien eine wichtige Rolle im Zellulose-Abbau spielten, was eine gewisse Redundanz zwischen unterschiedlichen mikrobiellen Gruppen hinsichtlich der genutzten Substrate zeigt, aber Unterschiede im Nährstoff-Bedarf.

Die Ergebnisse dieser Arbeit verdeutlichten einen klaren Zusammenhang zwischen Ressourcenverfügbarkeit und mikrobiellen Prozessen. Die Art der verfügbaren Substrate, sowie die Verfügbarkeit von labilem C und N übten starken Einfluss auf mikrobielle Prozessraten aus und beeinflussten auch die Zusammensetzung der mikrobiellen Gemeinschaften und ihre funktionellen Eigenschaften. Auswirkungen der mikrobiellen Gemeinschaftsstruktur auf mikrobielle Prozesse zeigten sich am deutlichsten beim Abbau von komplexen C-Substraten, wie pflanzlichen Zellwänden, und schwer abbaubarer organischer Substanz (Humus). Dies lässt darauf schließen, dass nur spezialisierte Gruppen von Mikroorganismen die physiologischen Fähigkeiten zum Abbau dieser Substanzen haben, während die Kapazität zur Nutzung von labileren Substraten, wie Protein, unter Mikroorganismen weiter verbreitet ist.

VI. APPENDIX

Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil

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Belowground carbon allocation by trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil

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Summary

- Plant seasonal cycles alter carbon (C) and nitrogen (N) availability for soil microbes, which may affect microbial community composition and thus feed back on microbial decomposition of soil organic material and plant N availability. The temporal dynamics of these plant–soil interactions are, however, unclear.
- Here, we experimentally manipulated the C and N availability in a beech forest through N fertilization or tree girdling and conducted a detailed analysis of the seasonal pattern of microbial community composition and decomposition processes over 2 yr.
- We found a strong relationship between microbial community composition and enzyme activities over the seasonal course. Phenoloxidase and peroxidase activities were highest during late summer, whereas cellulase and protease peaked in late autumn. Girdling, and thus loss of mycorrhiza, resulted in an increase in soil organic matter-degrading enzymes and a decrease in cellulase and protease activity.
- Temporal changes in enzyme activities suggest a switch of the main substrate for decomposition between summer (soil organic matter) and autumn (plant litter). Our results indicate that ectomycorrhizal fungi are possibly involved in autumn cellulase and protease activity. Our study shows that, through belowground C allocation, trees significantly alter soil microbial communities, which may affect seasonal patterns of decomposition processes.

Introduction

The seasonal cycles of plants and soil microbes in temperate forests are closely linked by the availability of nutrients and labile carbon (C). Plants affect C and nitrogen (N) availability for soil microbes as a result of competition for nutrients during active growth, exudation of labile C via roots and substrate input by litterfall (Wardle *et al.*, 2004; Bardgett *et al.*, 2005; Harrison *et al.*, 2008). Microbes, in turn, control nutrient availability for plants by carrying out a wide spectrum of decomposition processes (Schimel *et al.*, 2007; Schmidt *et al.*, 2007). Thus, seasonal variations in plant growth phases as well as in microbial community composition and physiology may strongly affect C and N availability, which in turn feed back on plant and microbial processes over the course of a year.

Microbial decomposition processes in soil have been shown to be highly sensitive to the availability of labile C and N (Schimel & Weintraub, 2003). For example, the degradation of humified soil organic matter (SOM) is thought to be energy limited, leading to very slow SOM decomposition rates in soils (Kuzyakov *et al.*, 2009). The input of labile

C by plant root exudation, however, may provide energy and enable microbes to degrade SOM to gain limiting nutrients (Paterson, 2009). Plant exudates may also fuel decomposition of other complex substrates, such as soil proteins (De Nobili *et al.*, 2001; Weintraub *et al.*, 2007) or litter (Subke *et al.*, 2004). Complementarily, many studies have shown that changes in N availability for soil microbes lead to alterations in decomposition rates of SOM and litter (Waldrop & Zak, 2006; Allison *et al.*, 2008; Keeler *et al.*, 2009). Together, these findings indicate that the availability of labile C and N controls the decomposition of complex substrates and thus the availability of nutrients for plants.

The mechanism by which C and N availability may control decomposition rates remains unclear. One possible explanation for this phenomenon may be the tight coupling between microbial community structure and function that has been proposed recently (Prosser *et al.*, 2007; Strickland *et al.*, 2009). As a result of the different nutrient demands and growth characteristics of specific microbial groups, altered C and N availability may favour the growth of certain microbial groups over others, thereby leading to microbial community shifts (Brant *et al.*, 2006). This may, in turn, strongly influence decomposition processes, as different microbial groups may exhibit different capacities to degrade high molecular weight substances, such as lignin, cellulose or humified SOM.

In temperate ecosystems, a strong effect of seasons on plant–soil interactions leads to seasonally variable C and N availabilities for both plants and microbes. As described above, C and N availability may be – together with abiotic factors – major drivers of microbial decomposition processes in soils. Microbial decomposition of SOM, however, represents a key driver of the forest's C and N cycle, which highlights the need for a better understanding of how this process is influenced by plant-mediated C and N availability. Unraveling the effects of aboveground–belowground relationships on ecosystem processes and function on seasonal time-scales still remains one of the major challenges in ecosystem ecology (Bardgett *et al.*, 2005). To improve our understanding of those relationships, it is, however, necessary to gain a deeper insight into the link among C and N availability, microbial community dynamics and microbial decomposition processes in a seasonal context.

The aim of this study was to explore this link over the course of 2 yr in a temperate forest. We hypothesized that the seasonal changes in microbial community composition and physiology are driven by C and N availability in addition to abiotic factors; and that microbial community composition affects specific decomposition processes mainly through the production of distinct sets of extracellular enzymes. We tested these hypotheses by analysing the seasonal patterns of nutrient availability, microbial community structure and decomposition processes in monthly to bimonthly measurements over a period of 2 yr. Additionally, we experimentally altered the C and N availability and C:N stoichiometry through N fertilization and tree girdling. Tree girdling has been shown to effectively cut off the translocation of photoassimilates from the canopy to the roots, thereby preventing the exudation of labile C from plant roots into the soil (Högberg *et al.*, 2001; Subke *et al.*, 2004; Scott-Denton *et al.*, 2006). To link microbial community composition to decomposition processes, we investigated whether seasonal or treatment-specific microbial community changes were related to changes in specific enzyme activities.

Materials and Methods

Study site

Our study site was located in a mature beech forest (*Fagus sylvatica* L.) *c.* 40 km south-west of Vienna, Austria (510 m asl). The age of the trees was on average 65 yr. The soil was a dystric cambisol (over flysh) with a pH of between 4.5 and 5.1 ($CaCl_2$). Organic C and total N content comprised 7.45% and 0.48% of dry soil, respectively. Despite the proximity to the city of Vienna, the site received an N input through atmospheric deposition of only 12.6 kg N ha^{-1} yr^{-1} (Kitzler *et al.*, 2006).

Experimental set-up

The selection of control and treatment plots followed a randomized block design. First, three blocks within an area of *c.* 5400 m^2 were selected based on a geobotanic characterization, such that each block had more or less homogenous vegetation and similar soil properties. Within each block, two control plots, two fertilization plots (5 × 5 m each) and one girdling area (20 × 20m) were chosen randomly. Two girdling plots were installed in the central 10 × 10 m of each girdling area. Each block thus contained two replicate plots of each treatment.

The fertilization treatment plots were fertilized once a month (after the soil sampling) with 29.7 g NH_4NO_3 dissolved in 2 l of water to give a final N fertilization rate of 50 kg ha^{-1} yr^{-1}. The fertilizer was evenly distributed on each plot by spraying. The first fertilizer application was on 9 May 2006, 1 month before the first sampling. On the same day, all trees within each girdling area were girdled by ripping off a 20-cm strip of bark around the stem at a height of *c.* 1.50 m. Understory plants, which consisted mainly of beech seedlings, a few herbs and sedges, were removed from all plots (girdling, control and fertilization). Understory plants were again removed in the following spring.

Soil sampling

Soil samples were taken from the upper 5 cm of mineral soil (A horizon). Soils from control plots were sampled every

month between June 2006 and June 2008 (24 samplings). Soils from fertilized and girdled plots were sampled every 2 months, except for June and July 2007 (which were both sampled; 13 samplings in total). Four subsamples were taken from each of the six replicate plots for treatments and controls and pooled to give one replicate. Sampling was based on a predetermined sampling scheme in order to avoid sampling of already disturbed soil. Soil samples were carefully sieved (2 mm), freed from visible roots by hand-picking and kept at 4°C until further processing. All extractions were carried out on fresh soils within 4 d after sampling.

Fine-root biomass

Fine-root biomass was determined 4, 14 and 28 months after girdling. The determination of root biomass 4 months after girdling was conducted in a parallel girdling experiment at the same study site, which started in May 2008 (three new girdling areas, with the same outline and experimental set-up as the girdling experiment described above). Thus, root biomass was determined in July 2007 (14.5 months after girdling) and September 2008 (4 and 28 months after girdling). Five soil cores (7 cm in diameter and 14.5 cm in height) were taken from the upper mineral soil (A horizon) of each of the six replicate control and girdled plots. For each soil core, fine roots (diameter < 1 mm) were carefully separated from coarse and visibly dead roots, thoroughly washed and weighed to determine fine-root biomass. Fine roots were pooled for each replicate plot. Mycorrhizal colonization was determined from aliquots of pooled samples.

Determination of mycorrhizal root colonization

Entire root subsamples were evenly dispersed in a 9-cm-diameter Petri dish, and root length was measured using a line intersect method at ×30 magnification (Newman, 1966). The degree of mycorrhizal root colonization was determined by counting all ectomycorrhizal root tips in four randomly selected squares making up 11% of the total area of the Petri dish.

Soil moisture and temperature

Soil moisture was detected gravimetrically in soil samples. Soil temperature was measured using Pt100 sensors (Kucera Company, Brno, Slovakia) at 5 cm depth and data were collected every 0.5 h. The soil temperature data presented are means of soil temperature measurements collected during the 6 d time period preceding the sampling date.

Dissolved organic carbon (DOC) and total N

DOC and total dissolved N (dN) were measured in soil water extracts (2 g of fresh soil was extracted with 20 ml of analytical grade water; extracts were stored at × 20°C) using a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V; Shimadzu, Vienna, Austria).

Phospholipid fatty acids (PLFAs)

PLFAs were analyzed using a modified procedure described in Frostegard *et al.* (1991). Samples were processed within 1 d after sampling. Total lipids were extracted with chloroform/methanol/citric acid buffer (0.15 M), pH 4.0 (1 : 2 : 0.8, v/v/v). Neutral lipids were separated from phospholipids on silica columns (Supelco, LC-Si SPE, Vienna, Austria) by elution with chloroform, acetone and methanol. After adding methyl-nonadecanoate (19:0) as an internal standard, phospholipids were converted to fatty acid methyl esters (FAME) by alkaline methanolysis. Dried FAMEs were redissolved in isooctane and analyzed by gas chromatography (HP G1530A, Agilent, Vienna, Austria) on a DB23 column (Agilent, Vienna, Austria). A bacterial FAME mix (Supelco) was used as qualitative standard. Concentrations of FAMEs were calculated using the internal standard (19:0) peak as a reference.

We used i15:0, a15:0, i16:0, i17:0 and a17:0 as indicators for Gram-positive bacteria, 18:1ω7, cy17:0, 16:1ω7, 16:1ω9, cy18:0, cy19:0 and 16:1ω5 as indicators for Gram-negative bacteria, and the sum of Gram-positive and Gram-negative biomarkers together with 18:1ω5, 17:0, 15:0, 17:1ω6 and 17:1ω7 as a measure for total bacteria. The biomarkers 18:2ω6,9, 18:1ω9 and 18:3ω3,6,9 are frequently used as fungal markers (Hill *et al.*, 2000; Leckie, 2005; Högberg, 2006; Joergensen & Wichern, 2008). However, as they have also been found to occur in plants (Zelles, 1997; Laczko *et al.*, 2004) we measured the concentrations of these biomarkers in beech roots and calculated that the possible contribution of root-borne PLFAs (based on fine-root biomass measurements and the assumption that 95% of roots were removed by sieving) to our soil samples was ≤ 0.61% for 18:2ω6 and 18:1ω9 (or ≤ 0.31% in girdled plots) and up to 4.1% for 18:3ω3,6,9 (or 1.2% in girdled plots). Thus, the observed fine-root loss in girdled plots would account for 1.25, 0.79 and 6% of the observed decrease in 18:1ω9, 18:2ω6 and 18:3ω3,6,9, respectively (Kaiser *et al.*, 2010).

We used the sum of all PLFAs described above together with the PLFAs 20:2ω6,9 (protozoa), 10Me16:0 (actinomyceta) and 14:0, i14:0, 16:0, 19:1ω8, i17:1ω8, 18:0, 16:1ω11, 16:1ω6, 19:1ω7, 20:0, 14Me15:0 and 20:1ω9, which are not specific for any microbial group, as a measure of total microbial biomass.

Extracellular enzymes

Potential extracellular enzyme activities were measured using microplate fluorometric and photometric assays. All

activities were measured within 48 h after sampling of soils. One gram of sieved soil was suspended in 100 ml of sodium acetate buffer (100 mM, pH 5.5) and ultrasonicated at low energy (Stemmer *et al.*, 1998; Marx *et al.*, 2001). β-1,4-Cellobiosidase ('cellobiosidase'), β-1,4-N-acetylglucosaminidase, chitinase/lysozyme ('chitinase') and leucine amino-peptidase ('peptidase') were measured fluorimetrically (Marx *et al.*, 2001; Saiya-Cork *et al.*, 2002). Two hundred microliters of soil suspension and 50 μl of substrate (4-methylumbelliferyl-β-D-cellobioside, 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide, 4-methylumbelliferyl-β-D-N,N′,N″-triacetylchitotrioside and L-leucine-7-amido-4-methyl coumarin, respectively) were pipetted into black microtiter plates in three analytical replicates. Methylumbelliferyl (MUF) was used for calibration of cellobiosidase, N-acetylglucosaminidase and chitinase, whereas aminomethylcoumarin (AMC) was used for calibration of leucine amino-peptidase. Plates were incubated for 140 min in the dark and fluorescence was measured at 450 nm emission at an excitation at 365 nm (using a Tecan Infinite M200 fluorimeter, Werfen, Austria).

Different enzymes with different abilities to cope with steric hindrance may be involved in the degradation of polymers, such as chitin. We therefore assayed chitinases with two types of substrate: 4-methylumbelliferyl-β-D-N,N′,N″-triacetylchitotrioside, consisting of three units of N-acetyl-β-D-glucosaminide (component of chitin), and 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide, consisting of only one unit.

Phenoloxidase and peroxidase activities were measured photometrically according to standard methods (Sinsabaugh *et al.*, 1999), with small modifications. Subsamples were taken from the soil suspension (see above) and mixed with a 20 mM L-3,4-dihydroxyphenylalanin (L-DOPA, Sigma-Aldrich, Vienna, Austria) solution (1 : 1). Samples were shaken for 10 min and centrifuged, and aliquots were pipetted into microtiter plates (six analytical replicates per sample). Half of the wells additionally received 10 μl of a 0.3% H_2O_2 solution for measurement of peroxidase. Absorption was measured at 450 nm at the starting time-point and after 20 h. Enzyme activity was calculated from the difference in absorption between the two time-points divided by the molar extinction coefficient, which had been determined in a preliminary experiment.

Actual extracellular enzyme activities

'Actual' enzyme activities were measured without substrate and buffer additions, but with the addition of toluene to inhibit microbial uptake of enzymatic products, leading to their accumulation in the soil suspension (Boschker *et al.*, 1995; Watanabe & Hayano, 1995; Lipson *et al.*, 1999; Weintraub & Schimel, 2005). This method has been used before to determine actual protease activities (accumulation of amino acids; Lipson *et al.*, 1999; Weintraub & Schimel, 2005) and to determine polysaccharide degradation (accumulation of glucose and xylose; Boschker *et al.*, 1995). We measured both activities in the same assay: 8 g of fresh soil was mixed with 80 ml of distilled water and 0.8 ml of toluene in a glass jar and shaken for 4 h. Subsamples for the detection of amino acids and glucose were sampled after 0.5 and 4 h. For measurement of amino acid production, subsamples were centrifuged and supernatants were mixed with the same volume of a solution containing 0.11 M trichloroacetic acid, 0.22 M sodium acetate and 0.33 M acetic acid, to stop further enzyme activity (Weintraub & Schimel, 2005). Samples were analyzed for amino acids using a photometric assay with ninhydrin (Moore, 1968). For measurement of glucose production, subsamples were centrifuged twice at 15 000 ***g*** to remove soil particles and microbes and were incubated at 100°C for 15 min to stop enzyme activities. Samples were then analyzed for glucose by HPLC (ICS-3000; CarboPac PA20 column; 10 mM NaOH, with pulsed amperiometric detection, DIONEX, Vienna, Austria). In a preliminary experiment, the accumulation of both amino acids and glucose was shown to be linear up to 6 h. Glucose accumulation in the soil solution was probably caused by cellulase and/or amylase activity (Boschker *et al.*, 1995). We detected some accumulation of cellobiose and maltose in the soil solution, but concentrations were much lower than that of glucose, indicating that these intermediate products were rapidly further degraded. Thus, we used glucose production as a proxy for the combined action of cellulose and amylase. We ascribed accumulation of amino acids in the soil solution to protease activity (Weintraub & Schimel, 2005).

Statistics

We carried out a canonical correspondence analysis (CCA; Anderson & Willis, 2003; Gonzalez *et al.*, 2008) on a data set of 11 samplings (bimonthly from August 2006 to May 2008; August 2007 had to be excluded because of missing data for phenoloxidase and peroxidase activity (n = 195)). A total of 37 individual PLFA biomarkers were used as the community matrix (nmol PLFAs g^{-1} dry soil), whereas different sets of constraining variables (abiotic conditions, C and N pools and microbial processes) were used to compare the influence of different groups of environmental parameters on microbial community composition. The contribution of constrained variability to total community variability (= fraction of variability explained by the parameter) in each analysis was used as a measure of the influence of the respective parameter set on community variation (used in Table 2). Results from a CCA conducted with all parameters (except glucose production, which was not available for the first two samplings) as the constraining matrix are presented graphically in more detail (see Fig. 6 below). We conducted a linear regression analysis (ordinary

least square regression) between all individual PLFA biomarkers and all measured soil parameters for the 2-yr sampling period. All statistical analyses were performed in R 2.8.1 (http://www.r-project.org/, R-package *vegan*).

Results

Climate and abiotic conditions

Temperature and precipitation patterns were different between the two sampling years (June 2006 to May 2007 and June 2007 to May 2008). The first autumn and winter period (September 2006 to January 2007) was on average 2.2°C warmer than the second autumn/winter, and exceptionally dry, with almost no precipitation and no snow cover. By contrast, the second sampling year was characterized by continuous snow cover from November to March. Soil water content ranged from 25 to 30% between October and January of the first sampling year, and from 30 to 40% in the same period of the second year (Fig. 1b). In both years, soil water content was lowest in August (23%). Soil temperature was *c.* 1.3°C higher between September and May of the first year compared with the second year (Fig. 1a).

Girdling affected soil water content significantly. The magnitude of this effect, however, depended on season. Soil moisture was significantly higher in girdled plots from September to December of both years (by 5–10%), and in spring and summer of the second year. No difference, however, was observed between January and March of both years (Table 1, Fig. 1b). Fertilization did not affect soil water content. There was no difference in soil temperature between treatments and controls.

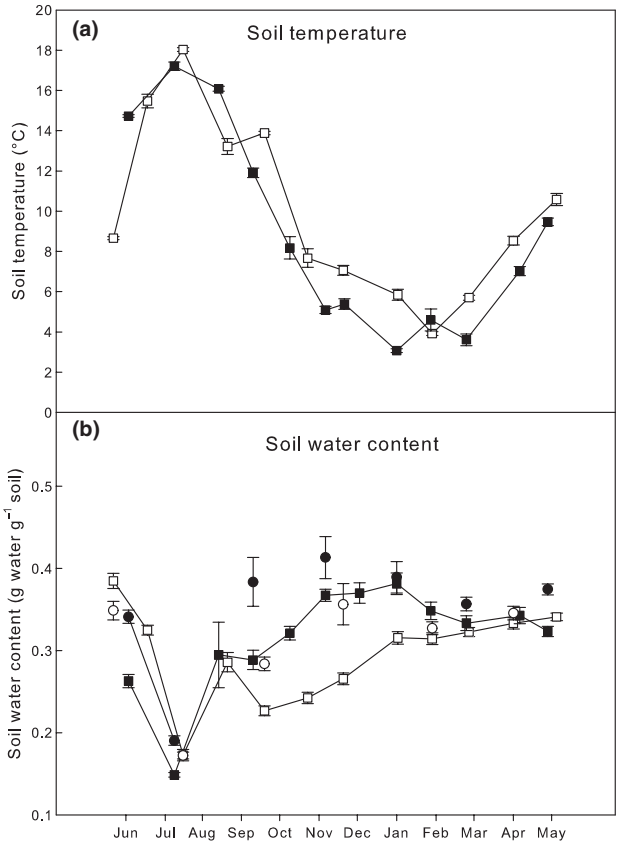

Fig. 1 (a) Mean soil temperature (at 5 cm depth) during the two sampling years (2006/2007 and 2007/2008). Each data point represents the average of the daily mean soil temperatures on the 7 d preceeding each sampling date (for all treatments: open symbols, year 1; closed symbols, year 2). (b) Gravimetric soil water content (0–5 cm depth) for control and girdled plots (squares, controls; circles, girdled plots) during the two sampling years. Error bars indicate 1 SE (n = 6).

Effect of girdling on tree vitality

In the first year, leaf senescence started *c.* 3 wk earlier in girdled trees. In the following spring, however, girdled trees still exhibited a full flush of leaves. The amount of leaf litter in the second sampling year was similar in all plots, although litter fall occurred earlier in girdled plots (34% of total leaf litter fall in girdled plots but only 13% in control plots took place before the end of September 2007; Supporting Information Fig. S1). In spring 2008 (2 yr after girdling), 40% of girdled trees did not produce new leaves.

Fine-root biomass and ectomycorrhizal colonization

Fine-root biomass in control plots was *c.* 1–1.2 g dry fine roots dm^{-3} soil in the upper 14.5 cm of soil. We observed no difference in fine-root biomass or ectomycorrhizal root tip colonization between control and girdled plots 4 months after girdling (Fig. 2). Fine-root biomass decreased by 45 and 60% in girdled plots 14 and 28 months after girdling, respectively. Mycorrhizal root colonization was reduced by 60% 14 months after girdling, but only by 27% 28 months after girdling. However, because of the smaller amount of vital roots at the latter time-point, the total amount of mycorrhizal root tips per cm^3 soil was decreased by *c.* 70% for both 14 and 28 months after girdling (Fig. 2).

DOC and N levels

In both years, DOC content in soil water extracts was low in early autumn (September to October), increased in late autumn (November to December) and decreased again to low values between January and April, followed by a sharp increase in May (Fig. 3b). Thus, autumn and winter dynamics were similar between the two sampling years, probably indicating a general seasonal trend. By contrast, we could not observe similar DOC dynamics in early and late summer of the two years. Tree girdling decreased DOC in soil water extracts predominantly in July of both years

Table 1 Significance of the effects of girdling, fertilization and sampling month on various soil parameters derived from an analysis of variance (ANOVA)

	Year 1						Year 2					
	Girdling			Fertilization			Girdling			Fertilization		
Soil parameter	m (df = 5)	g (df = 1)	g × m (df = 5)	m (df = 5)	f (df = 1)	f × m (df = 5)	m (df = 6)	g (df = 1)	g × m (df = 6)	m (df = 6)	f (df = 1)	f × m (df = 6)
Soil water content	***	***	***	***	ns	ns	***	***	*	***	ns	ns
Cellobiosidase	***	ns	ns	***	ns	ns	***	ns	°	***	ns	ns
Chitinase	***	°	ns	***	ns	ns	***	**	ns	**	ns	ns
N-Acetylglucosaminidase	***	ns	ns	***	ns	ns	***	ns	**	***	ns	ns
Leu-peptidase	***	ns	ns	***	ns	ns	***	ns	***	***	ns	ns
Phenoloxidase[a]	***	°	***	***	ns	ns	***	ns	***	***	*	***
Peroxidase[a]	***	***	**	***	ns	ns	***	ns	***	***	***	***
Actual protease[b]	***	***	ns	**	ns	ns	***	***	°	***	ns	*
Glucose production[c]	***	***	**	***	ns	ns	***	**	***	***	°	ns
DOC	**	ns	ns	**	ns	ns	***	ns	ns	***	ns	*
Total dissolved N (dN)	***	***	***	***	*	ns	***	***	***	***	**	ns
DOC: dN	***	***	***	***	***	**	***	***	***	***	**	**
CCA1	***	ns	ns	***	*	***	***	ns	ns	***	ns	°
CCA2	*	***	**	ns	ns	ns	**	***	ns	*	*	ns

Presented are levels of significance resulting from factorial ANOVAs (with month and treatment as factors) conducted separately for the girdling and fertilization treatments, and for each year of the experiment. DOC, dissolved organic carbon; m, month of sampling; g, girdling; f, fertilization; g × m and f × m, interactions of month and treatment. The number of replicates for the first and second years was $n = 72$ (six samplings) and $n = 84$ (seven samplings), respectively. Degrees of freedom (df) for each factor are indicated in the table headings, with the following exceptions. [a]$n = 72$ and df (m and m × treatment) = 5 for year 2; [b]$n = 60$ and df (m and m × treatment) = 4 for year 1; [c]$n = 48$ and df (m and m × treatment) = 3 for year 1. CCA1, CCA2, sampling scores of the first two axes of the canonical correspondence analysis (Fig. 6, Supporting Information Fig. S2).
Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; °, $P < 0.1$.
ns, not significant.

(−21.7% ($P = 0.058$) and −20% ($P = 0.044$), respectively), but did not affect DOC during autumn and winter (Fig. 3b). Total (= organic and inorganic) dissolved N in soil water extracts peaked around mid-summer (August) in both years, followed by a sharp decrease in autumn to low levels in winter (Fig. 3a). Levels of dissolved N were significantly enhanced by fertilization and girdling, on average (over the whole sampling period) by 28 and 170%, respectively ($P < 0.001$ and $P < 0.05$, respectively; Fig. 3, Table 1).

Soil enzyme activities

Soil enzyme activities were high in spring, late summer and autumn, and low in mid-summer and winter (Fig. 4). The spring peak was highly synchronous for all measured enzymes (in 2007), starting with fairly low levels in March and increasing to maximum levels in June, followed again by a summer decline. The second ('autumn') peak occurred at different times for different enzymes: (potential) phenoloxidase and peroxidase showed maximum activities already in late summer (around September), whereas glucose production (by actual cellulase/amylase activity; Fig. 4g) and actual protease activity (Fig. 4h) had their autumn maximum around November. At this time, phenoloxidase and peroxidase activities had already dropped to very low levels (Fig. 4e,f).

We found no significant effect of girdling or fertilization on enzymes measured using fluorescent substrates (chitinase, cellobiosidase, N-acetylglucosaminidase and peptidase) during the first year (Table 1, Fig. 4a–d). In the second year, chitinase was found to be significantly decreased in girdled plots (Table 1, Fig. 4a).

'Actual' protease and cellulase activities were markedly reduced in girdled plots, whereas phenoloxidase and peroxidase activities were significantly enhanced (Fig. 4e–h, Table 1). These shifts in activities occurred already in the first summer after girdling. Fertilization affected phenoloxidase and peroxidase activity only in the second year (Table 1, Fig. 4e,f).

Microbial biomass

The annual course of the amount of total PLFAs, as a measure of microbial biomass, was similar in the two sampling years in control plots, apart from a small time shift (Fig. 5a). Microbial biomass showed two phases of lower values during the course of a year: the first in June (July in

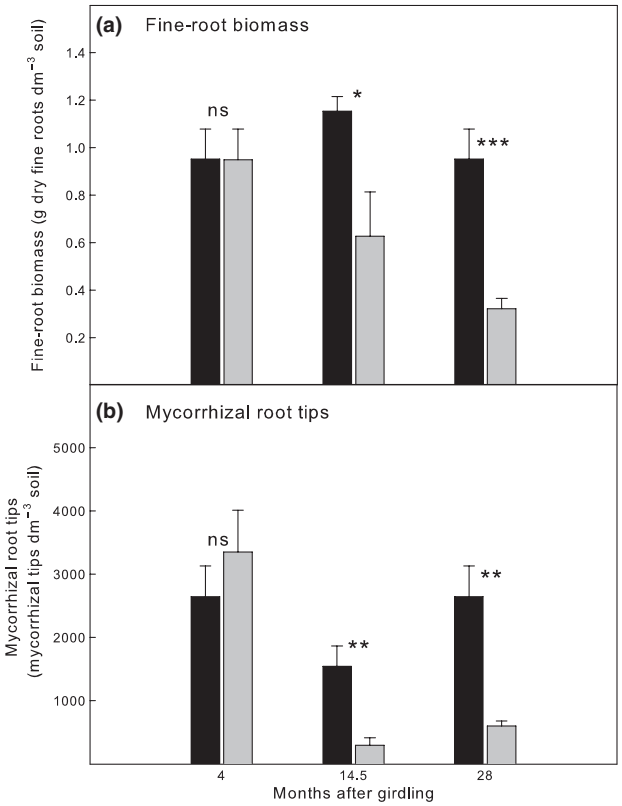

Fig. 2 Dry fine-root biomass (a) and ectomycorrhizal root tips (b) per dm^{-3} of soil in control and girdled plots at 4, 14.5 and 28 months after girdling. Black bars, controls; gray bars, girdled plots. Error bars indicate 1 SE (n = 6). Asterisks indicate statistical significant differences between control and girdled plots for each time-point (ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

the second year) and the second in February (March in the second year). In both years we observed a sharp increase of biomass in spring and a period of high values in autumn (Fig. 5a, Table S1).

Girdling decreased the total amount of PLFAs by 15% on average between July 2006 and January 2008 (2–20 months after girdling). In the same time period, the sum of bacterial biomarkers was decreased by *c.* 14%, the amount of the fungal biomarker 18:1ω9 by 16% and the fungal biomarker 18:2ω6,9 by as much as 51% (Fig. 5).

Fungi are thought to contain smaller amounts of PLFAs per g biomass compared with bacteria because of a higher ratio of cell volume to cell surface, which makes it difficult to estimate the real fungi to bacteria ratio from PLFA data (Joergensen & Wichern, 2008). Thus, the reduction of 51% of the 18:2ω6,9 biomarker may translate into a much greater reduction of the total microbial biomass than the 15% loss of total PLFAs suggests. It is worth noting that in girdled plots the seasonal trend was maintained for bacteria and the fungal biomarker 18:1ω9, but not for the fungal biomarker 18:2ω6,9, which had already started to decrease strongly 2 months after girdling (Fig. 5). Fertilization had no significant effect on the total amount of bacterial or fungal PLFA biomarkers.

Microbial community composition

We conducted a canonical correspondence analysis (CCA) in order to assess how far the variation observed in the microbial community composition was related to specific environmental parameters. By contrast to unconstrained multivariate analysis, CCA displays only the part of the variation in the community data that can be explained by the used constraints. Our analysis revealed that soil temperature and soil water content were related to 20% of the PLFA variability, whereas labile C and N (DOC and dissolved N) could only explain 4.3% of the total community variability (Table 2). Enzyme activities, however, were linked to 24% of the variability in the community matrix. Of all enzymes, phenoloxidase, peroxidase and peptidase were most strongly related to community structure (19.9%), whereas other enzymes explained much smaller parts of the variance (e.g. N-actyl-glucosaminidase, chitinase and cellobiase only 5.4%; Table 2).

Altogether, *c.* 36% of the variability of the microbial community matrix throughout seasons and treatments could be related to a combination of enzymatic activities and environmental parameters (Fig. 6, Table 2). Of the constrained variability, CCA axis 1 (CCA1) accounted for 72.2%, and CCA axis 2 (CCA2) for 12.4%. Both CCA1 and CCA2 were significant ($P < 0.001$; permutation test). We found significant effects of seasons and girdling on microbial community composition along these two axes (Fig. 6, Table 1). Different sampling months were mainly separated on CCA1 whereas control and girdled plots could be separated on CCA2 (Fig. 6b). No general trend for specific microbial groups (Gram-negative bacteria, Gram-positive bacteria, all bacteria, fungi) was observed along CCA1, indicating that seasonal microbial community shifts were caused by changes within all groups. The community shift along CCA2 (reflecting the effect of girdling) was dominated by the influence of two fungal biomarkers (18:2ω6,9 and 18:3ω3,6,9) and one bacterial biomarker (18:1ω5), which were negatively correlated to that axis (indicating reduced abundance of these markers in samples from the girdling treatment). The influence of the third fungal biomarker (18:1ω9) was comparably low (Fig. 6).

Of all sampling months, February of the first year and November of the second year had the highest scores on CCA1 (Supporting information Fig. S2). Both were the first months in which winter conditions (high soil moisture and low soil temperature) occurred in the respective year. Spring and summer months were characterized by lower scores (Fig. S2). Soil temperature and soil water content as well as peroxidase and peptidase activities exhibited a strong effect on CCA1, indicating that they were closely related to

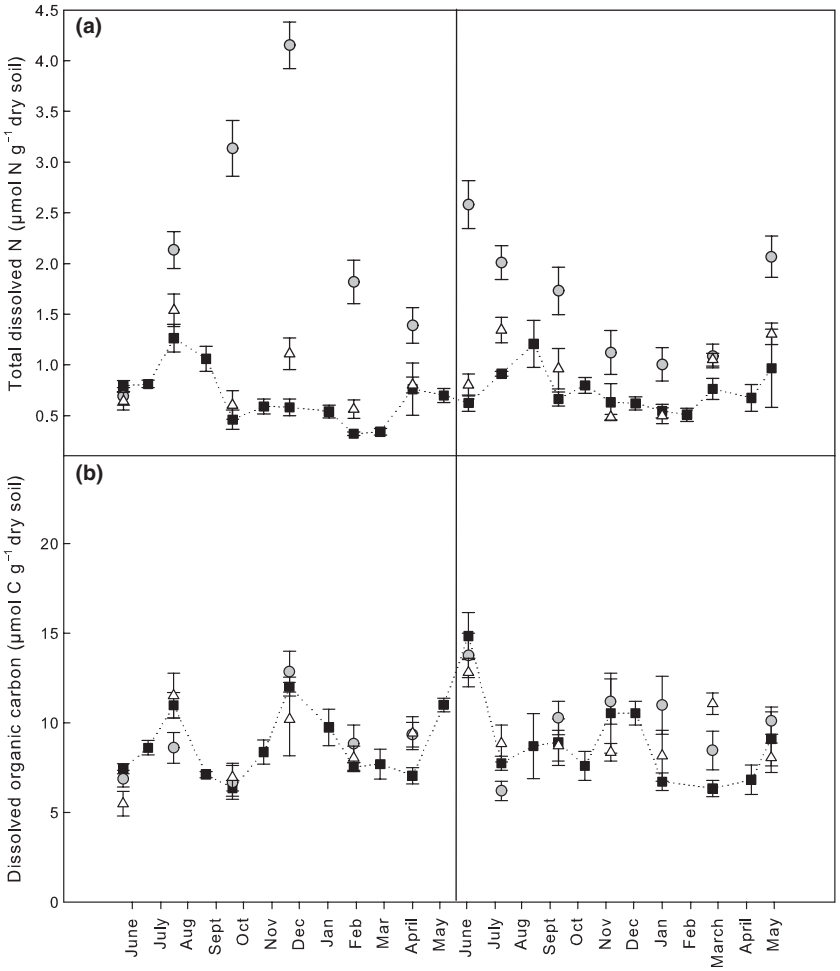

Fig. 3 Total dissolved nitrogen (a) and dissolved organic carbon (b) in soil water extracts over the course of the two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots; dotted lines connect control values to show the seasonal trend). All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n = 6).

seasonal variation of microbial community composition (Fig. 6).

A regression analysis of each individual biomarker with each microbial process revealed interesting links between microbial processes and community structure (Table 3). The results of this regression analysis showed that enzymatic activities can be separated into three groups, each of them relating to a specific set of individual PLFA biomarkers. First, phenoloxidase and peptidase activities were correlated with peroxidase activity and all three enzymes were correlated to the same same set of individual PLFAs, including the majority of Gram-positive biomarkers (i15:0, a15:0, i16:0), certain Gram-negative biomarkers (16:1ω7, 16:1ω9) and a few general bacterial biomarkers (14:0, 16:1ω11) (Table 3). Secondly, cellobiosidase, N-acetylglucosaminidase and chitinase were correlated with each other. Overall, these enzymes did not correlate with any specific PLFA biomarker, with the exeption of N-acetylglucosaminidase and chitinase which were correlated to the microbial biomass (sum of all PLFAs). The third group of enzyme activities, comprising 'actual' protease and cellulase/amylase activities, were not correlated to any bacterial biomarker, but – together with chitinase activity – to the fungal biomarkers 18:2ω6,9 and 18:3ω3,6,9 (Table 3). There was no correlation of one of the fungal biomarkers with peroxidase or phenoloxidase activity. However, when the regression analysis only for girdled plots was examined, a strong correlation (r^2 = 0.54, P < 0.005) between 18:2ω6,9 and phenoloxidase activity was found (Table S2).

Discussion

Tree roots provide an important source of easily assimilable C for soil microbes and allow the establishment of a specific microbial community (Brant *et al.*, 2006). In temperate forests, an important part of this community comprises ectomycorrhizal fungi (Read & Perez-Moreno, 2003; Jones *et al.*, 2004), but other microbial groups also benefit from this source of C (Butler *et al.*, 2003). Eliminating belowground C allocation by tree girdling in our study had a twofold effect on C and N availability for microbes. First, it led to decreased DOC contents in the soil during the summer. This effect was similar to that of other girdling studies which also found small season-dependent effects of girdling

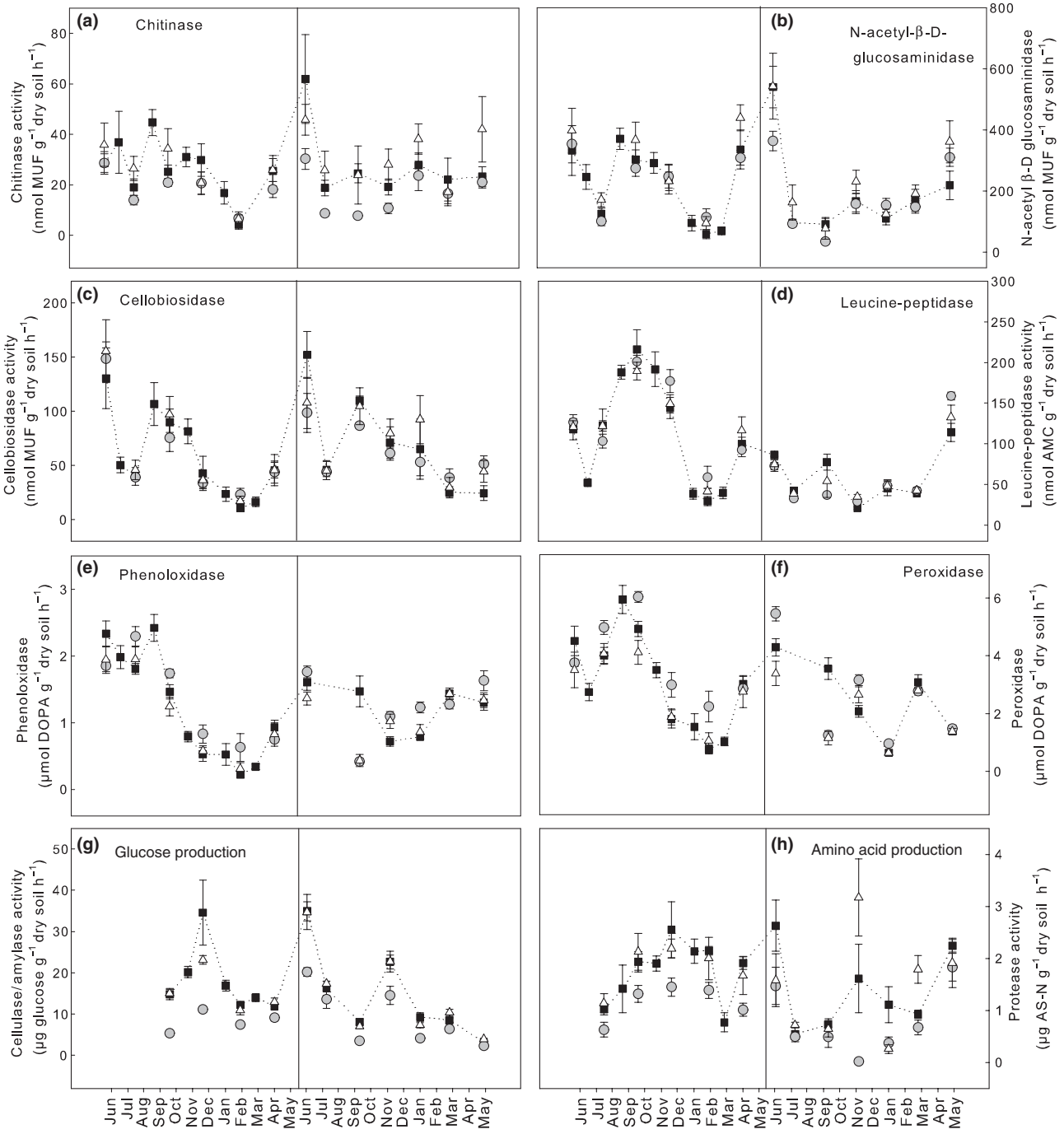

Fig. 4 Soil extracellular enzyme activities in control, fertilized and girdled plots throughout the 2-yr sampling period (squares, controls; circles, girdled plots; triangles, fertilized plots; dotted lines connect control values to show the seasonal trend). (a–f) Potential activities (activities measured using fluorometric and photometric methods after addition of a specific substrate). (g, h) Actual enzyme activities (no substrate added). All data are plotted on the actual sampling date; ticks are for the 15th day of each month. MUF, Methylumbelliferyl; AMC, Aminomethylcoumarin; DOPA, L-3,4-dihydroxyphenylalanin; AS-N, Amino acid-nitrogen. Error bars indicate 1 SE (n = 6).

on DOC contents in soil water extracts (Weintraub *et al.*, 2007; Zeller *et al.*, 2008). As DOC is a labile pool with fast turnover times, its absolute amount may provide little information about substrate supply to microbes. However, we also observed a reduced microbial biomass and a strong decline of soil CO_2 efflux (B. Kitzler *et al.*, unpublished), confirming that the availability of easily assimilable C was substantially depleted in the girdling treatments. By contrast, N concentrations were strongly enhanced in the soil water of girdled plots, presumably as a result of reduced

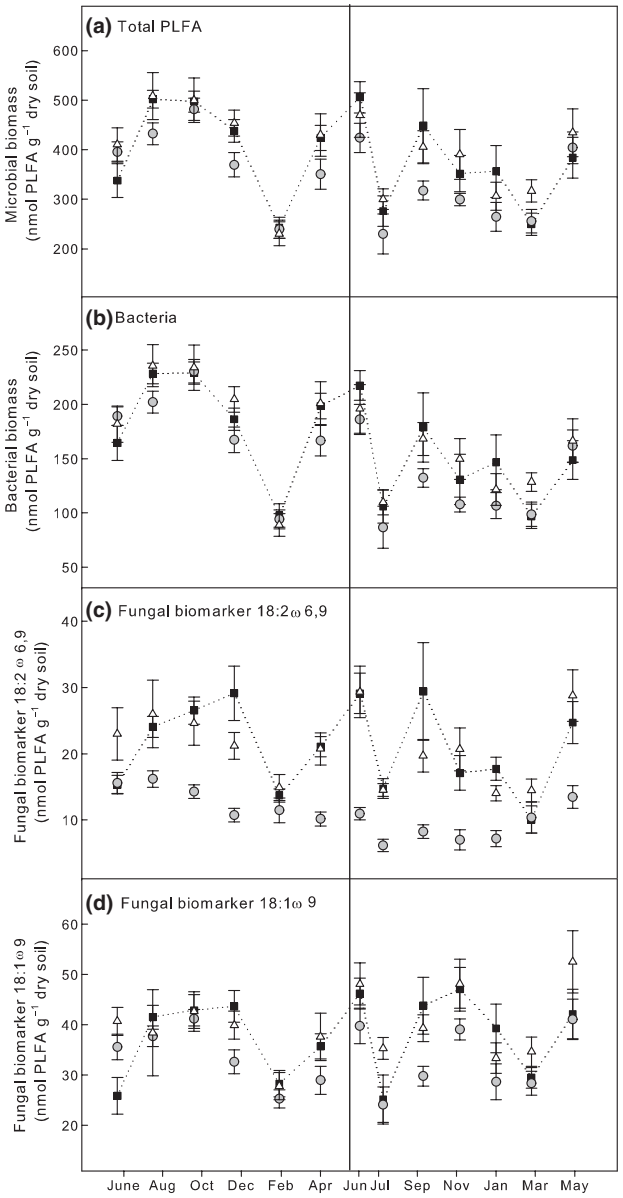

Fig. 5 Total phospholipid fatty acid (PLFA; a), bacterial (b) and fungal (c, d) biomarkers in control, girdled and fertilized plots over a period of 2 yr (squares, controls; circles, girdled plots; triangles, fertilized plots; dotted lines connect control values to show the seasonal trend). All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate 1 SE (n = 6).

plant N uptake caused by reductions in mycorrhizal hyphae and fine roots in the soil (Jordan *et al.*, 1998). Increased dissolved inorganic N concentrations in response to girdling have also been found in other studies (Weintraub *et al.*, 2007; Zeller *et al.*, 2008; Dannenmann *et al.*, 2009) and may affect microbial community composition in addition to the loss of root C input.

The effect of girdling on soil C and N availability led to substantial changes in microbial biomass and community composition. Although only a small proportion of the bacterial community (15%) was affected, the fungal biomarker 18:2ω6,9 was reduced by 51%. This is consistent with other girdling studies, which showed a strong decline of the fungal biomarker 18:2ω6,9, but almost no response of 18:1ω9. Compared with other types of fungi, mycorrhizal fungi depend to a much higher degree on belowground C allocation by trees. It is therefore safe to assume that the reduction of fungal biomarkers after girdling was related mainly to the reduction of mycorrhizal fungi, as has also been suggested by others (Högberg, 2006; Högberg *et al.*, 2007; Yarwood *et al.*, 2009). Surprisingly, we found a strong decrease of 18:2ω6,9 just 2 months after girdling (Fig. 5), although we did not observe any decrease of mycorrhizal root colonization 4 months after girdling (Fig. 2). The latter is consistent with the results of another beech girdling experiment (Dannenmann *et al.*, 2009), in which girdling had no effect on mycorrhizal root colonization in the first year. In our study, however, mycorrhizal root colonization decreased by as much as 60% 14 months after girdling (Fig. 2). Our results suggest that reducing the input of labile root C initially resulted in a strong decrease of the mycorrhizal extramatrical hyphal network in the soil within the first 2 months, followed only later by a decrease in ectomycorrizal root tips and a loss of fine-root biomass.

Potential activities of cellobiosidase, N-acetylglucosaminidase and peptidase were not significantly affected by girdling, demonstrating that they were not linked to root exudations. These enzymes (measured using relatively

Table 2 Relationship between different soil parameters and soil microbial community composition

Set of constraints	Explained fraction of variability (%)
Soil temperature and soil water content	20.3
DOC and dN	4.3
N-acetyl-β-D-glucosaminidase, chitinase and cellobiosidase	5.4
Peptidase	12.1
Phenoloxidase and peroxidase	15.9
Phenoloxidase, peroxidase and peptidase	19.9
Actual protease and cellulase/amylase*	6.3
All enzymes	24.2
All parameters	36.0

Presented are the contributions of constrained variability to total community variability by canonical correspondence analysis (CCA) conducted with different sets of constraining variables. CCA was based on data from bimonthly samplings of control, fertilized and girdled plots between August 2006 and May 2008 (with the exclusion of August 2007 because of missing peroxidase and phenoloxidase data; 11 samplings; n = 195). Additionally, data for July 2006 are missing for cellulose/amylase actitivity (*; 10 samplings; n = 177). DOC, dissolved organic carbon; dN, total dissolved nitrogen.

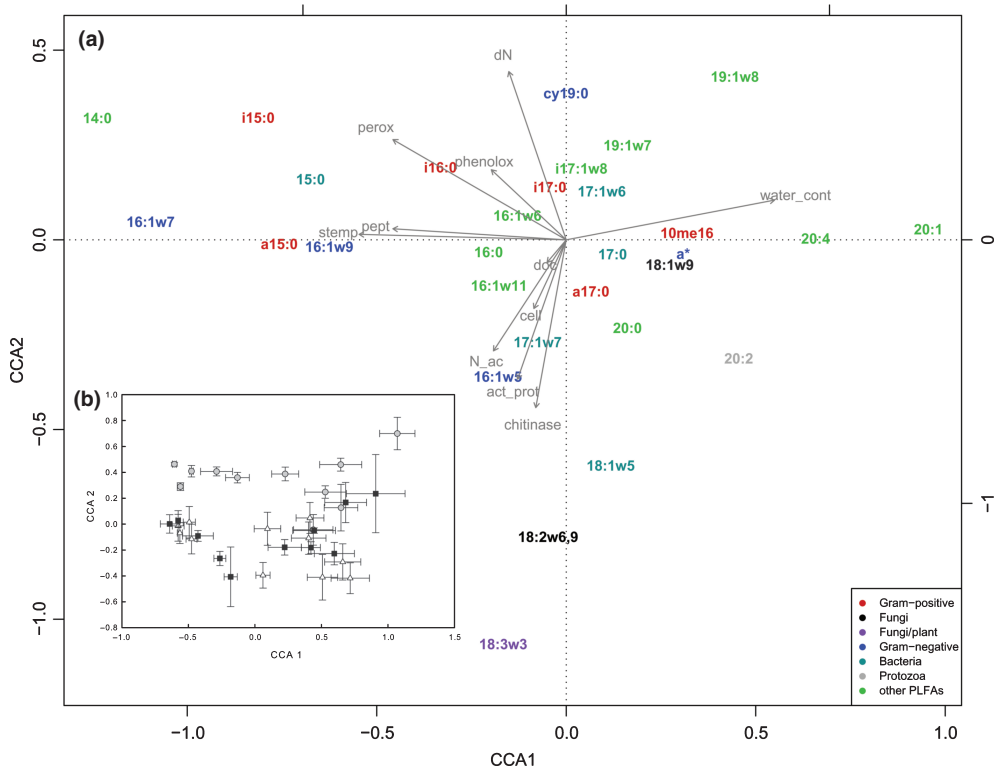

Fig. 6 Relationship between microbial community composition and various soil parameters analyzed by canonical correspondence analysis (CCA). Phospholipid fatty acid (PLFA) data were used as the community matrix; environmental and microbial process data as a constraining matrix. The analysis was based on data from all samplings between 6 August and 8 May, except for 7 July. (a) The distribution of individual PLFA biomarkers (weighted scores) is shown on the first two axes. Additionally, the biplot scores of constraining variables depicting the influence of soil parameters are shown on these axes. Biomarkers are colored according to their allocation to specific microbial groups. a*, 18:1ω7 and cy17:0; N_ac, N-acetyl-β-D-glucosaminidase; cell, cellobiosidase; stemp, soil temperature; act_prot, actual protease; pept, leucine-peptidase. (b) The distribution of the bimonthly samplings for the two sampling years is shown along the first two axes. Circles, girdled plots; squares, control plots; triangles, fertilized plots. The contribution of constrained variability to total variability was 36%, of which CCA1 accounts for 74.8% and CCA2 for 12.5%. CCA1 and CCA2 were both significant ($P < 0.001$; permutation test). The distribution of bimonthly samplings in the course of time is shown in Supporting Information Fig. S2.

simple low-molecular-weight substrates) possibly represent a general trait of microbial groups other than mycorrhizal fungi and rhizosphere bacteria, which were not affected by girdling. In contrast to the above-mentioned enzyme activities, our results showed a clear decrease of actual cellulase and protease activities in girdled plots already in the first year, especially at times when these rates were at peak values in control plots; that is, in spring and autumn (Fig. 4). The diminishing of the spring peak in girdled plots indicates that this peak is linked to root exudates. Spring exudates may have accelerated the turnover of microbes, leading to a flush of proteins and carbohydrates (De Nobili *et al.*, 2001; Kuzyakov, 2002; Phillips *et al.*, 2008). The autumn peak in enzyme activity, in contrast, may be explained by the input of fresh litter at that time. Approximately 70% of N-rich low-order roots are thought to have turnover times of < 1 yr (Kielland *et al.*, 2006; Guo *et al.*, 2008; Pritchard & Strand, 2008) and a large proportion of fine-root mortality is usually found at the end of the growing season (Ruess *et al.*, 2003). Together with leachates from leaf litter, this peak of fine-root mortality in autumn may therefore have led to a large input of cellulose and protein into the soil and subsequently to increased glucose and amino acid production.

As there was no reduction in overall fine-root biomass in the first year of girdling, we assume that this input of fresh substrates did occur in control as well as in girdled plots. Nevertheless, girdling strongly reduced this autumn peak of glucose and amino acid production (by > 50%) in November of both years (Fig. 4) This reduction was correlated to a strong reduction of the autumn and winter peak of fungal biomarkers in girdled plots (Fig. 5b, Table 3), suggesting that mycorrhizal fungi may be closely involved in protease and cellulase/amylase activity in autumn. At the time when amino acid and glucose production rates exhibited their autumn peak (in November), (potential) phenoloxidase and peroxidase activities strongly decreased to very low values (Fig. 4). Thus, we observed a temporal shift in enzyme activities from an early autumn maximum of

Table 3 Linear regressions of phospholipid fatty acid biomarkers and various soil parameters

PLFA biomarkers \ Soil parameter		Water content	Soil temp	Cellob.	Chitin.	N-acet.	L-pept.	Ph.ox.	Perox.	Act. prot.	Gluc. p.	dN	DOC: dN
Gram-positive	i15:0		0.36				**0.52**	0.37	**0.56**				
	a15:0		0.30				0.50	0.33	0.47				
	i16:0		0.38				0.36	0.38	0.45				
	i17:0		0.36					0.27	0.29				
	a17:0		0.27										
Actinomycetes	10me16												
Gram-negative	18:1ω7												
	cy17:0												
	16:1ω7					0.27	0.40	0.30	**0.54**				
	16:1ω9		0.36				0.49	0.27	0.36				
	cy18:0						0.32	0.34					
	cy19:0												
	16:1ω5			0.28	0.36		0.35	0.31	0.29	0.28			
Bacteria	18:1ω5												0.27
	17:00												
	15:00		0.36				0.41	0.35	**0.55**				
	17:1ω6												
Fungi	18:2ω6				0.29					0.36	0.27		0.35
	18:1ω9												
F&P	18:3ω3				0.26					0.27	**0.57**		0.38
General	14:00		0.36				**0.55**	0.38	**0.58**				
	16:00		0.33				0.32	0.30	0.36				
	16:1ω11			0.28	0.32		0.41	0.31	0.33				
	16:1ω6		0.37		0.27		0.35		0.35				
	17:1ω6						0.25						
	18:00		0.26						0.28		0.28		
Groups	Gram+		0.36				0.47	0.36	0.50				
	Gram−												
	Bacteria		0.27				0.41	0.34	0.38				
	Fungi												
	All		0.26		0.35	0.30	0.37		0.34				
Processes	Cellob.			**1.00**	0.42	0.32							
	Chitin.			0.42	**1.00**	**0.62**					0.35		
	N-acet.			0.32	**0.62**	**1.00**				0.37			
	L-pept.						**1.00**		0.30				
	Ph.ox.		0.34					**1.00**	**0.52**				
	Perox.		0.47				0.30	**0.52**	**1.00**				
	Act. prot.					0.37				**1.00**			0.32
	Gluc. p.				0.35						**1.00**		

Presented are goodness of fit (R^2) of statistical significant ($P < 0.01$) relationships between parameters; empty fields indicate that the regression was not significant. Bold values show $R^2 > 0.5$. Regressions are based on data from control and girdling plots from all samplings over the 2-yr sampling period (mean values of each sampling). Cellob., cellobiosidase; Chitin., chitinase; N-acet., N-acetyl-glucosaminidase; L-pept., leucine-peptidase; Ph.ox., phenoloxidase; Perox., peroxidase; Act. prot., actual protease; Gluc. p., glucose production; dN, total dissolved nitrogen; F&P, fungi and plants; DOC, dissolved organic carbon; PFLA, phospholipid fatty acid.

oxidative enzymes to a late autumn maximum of hydrolytic enzymes. While the maxima of cellulase and protease activities can be readily explained by increased substrate inputs in late autumn (see the Discussion section), the reasons for the drop in oxidative enzymes by *c.* 70% from September to December are less clear. Our data suggest that it is not likely that this drop was driven by abiotic factors. For example, October 2006 exhibited warmer soil temperatures than September, although there was already a strong decrease (by 45%) in oxidative enzymes in this period (Fig. 4). Potential measurements of oxidative enzyme activities may, however, reflect the abundance of the microbes producing them, rather than the actual rates of these enzyme activities. Phenoloxidase and peroxidase catalyze the key reactions in the degradation of lignin and humified SOM and have to be carried out by specialist microbes (Hatakka, 2001; Baldrian, 2009). However, specialists for SOM degradation will only have an advantage over others when other sources

of nutrients (such as fresh organic matter (FOM)) are scarce (Fontaine *et al.*, 2003; Kuzyakov *et al.*, 2009; Paterson, 2009), which is possibly the case during summer. Plant root exudates may provide a constant energy supply at low N levels during summer, thereby creating optimal conditions for SOM degraders (Fontaine *et al.*, 2003). This situation may change fundamentally when N-rich substrates, such as dying fine roots and leaf litter leachates, appear in autumn. SOM-degrading microbes may then lose their competitive advantage over microbes decomposing FOM, explaining the rapid autumn and winter decrease of phenoloxidase/peroxidase.

Competition between microbial groups could also have been responsible for the shift of enzyme activities from hydrolytic enzymes to oxidative enzymes in girdled plots. Mycorrhizal fungi are known to dominate the rooted soil layers as a result of a competitive advantage gained through access to root C, whereas saprotrophic fungi are thought to be more competitive in the litter layer (Hobbie & Horton, 2007; Lindahl *et al.*, 2007). Although ectomycorrhizal fungi have been found to be able to produce cellulases, proteases and phenoloxidases (Luis *et al.*, 2005; Cullings & Courty, 2009), saprotrophic fungi are thought to possess a much greater capacity to produce peroxidases (Luis *et al.*, 2005; Baldrian, 2009; Cullings & Courty, 2009). Girdling greatly decreased the abundance of mycorrhizal fungi, thereby possibly also giving saprotrophic fungi a competitive advantage in the rooting zone. At the same time, girdling reduced easily assimilable C in soils, which may have led to starvation of microbes, while N availability increased to very high levels (Fig. 3). According to the current understanding of rhizosphere priming, this would not favour SOM degradation, because under such conditions there is no need to mineralize N and SOM degradation to gain C would be too energy demanding (Kuzyakov *et al.*, 2009; Paterson, 2009). Nevertheless, potential oxidative enzyme activities rose in girdling plots in our experiment, suggesting that, at least in the longer term, the reduced abundance of mycorrhizal fungi may have relieved the competitive disadvantage of slow-growing specialist decomposers (e.g. saprotrophic fungi) which possess the ability to degrade SOM by releasing peroxidases and phenoloxidases (Hobbie & Horton, 2007; Baldrian, 2009).

Alternatively, reducing C input from the host tree could have led to a shift within the ectomycorrhizal community towards species with a greater ability to produce SOM-degrading enzymes or to a physiological switch of ectomycorrhizal fungi towards increased saprotrophic activity (Buee *et al.*, 2007; Talbot *et al.*, 2008; Cullings & Courty, 2009). Different mechanisms have recently been suggested which may lead to the decomposition of SOM by mycorrhizal fungi; for example, the need for an alternative C source when supplies of photosynthetates from plants are low, or, by contrast, 'priming' with high amounts of photosynthetates, which may accelerate the growth and activity of ectomycorrhizal fungi (Courty *et al.*, 2007; Cullings *et al.*, 2008; Talbot *et al.*, 2008). Our results suggest that, if mycorrhizal fungi were able to switch to saprotrophy at low C supply (as indicated by increased phenoloxidase and peroxidase activities in girdled plots), it was apparently not very effective, as fungal biomass strongly decreased in girdled plots. Instead, it seems plausible that fungi were primed by tree C in control plots, in which they grew to high abundances from summer to autumn and were linked to high activities of cellulase and protease, both of which diminished in girdled plots. An increase of phenoloxidase and peroxidase activities after girdling was also found by Weintraub *et al.* (2007), who suggested that this may have been caused by increasing availability of dead fine-root biomass as a result of girdling. However, because of the rapid increase of oxidative enzymes in girdled plots just 2 months after treatment and the lack of a decrease in fine-root biomass in the first 4 months (Fig. 2), we rule out this explanation for our experiment.

Regression analysis of PLFAs and enzyme activities over all seasons revealed three 'groups' of enzymes, each related to a specific set of individual PLFA biomarkers (Table 3). Of all measured enzyme activities, the activities of phenoloxidase, peroxidase and leucine-peptidase were those linked to the largest part of the variation (20%) in the microbial community composition, whereas cellobiosidase, N-acetylglucosaminidase and chitinase showed the weakest relation to community composition (Tables 2, 3). The high number of significant correlations between PLFA biomarkers and enzymes suggests that the observed enzyme pattern was determined to a large extent by microbial community dynamics, rather than by individual physiological response of microbes to varying nutrient and energy availability. Correlations between phenoloxidase, peroxidase and individual PLFAs were stronger and also involved the fungal biomarker 18:2ω6,9 (R^2 = 0.54, P = 0.007; Table S2) when girdled plots were analyzed alone, indicating that more microbial groups were connected to these enzyme activities after girdling (Table S2).

Tree C allocation to the belowground microbial community has been shown to enhance the decomposition of recalcitrant C compounds (Ekberg *et al.*, 2007) and to result in a long-term net C loss from soil (Carney *et al.*, 2007; Dijkstra & Cheng, 2007). Carney *et al.* (2007) showed that elevated root C input altered the microbial community structure towards a higher proportion of fungi, which in turn led to greater priming of SOM decomposition by leaf litter. This indicates that SOM decomposition may be determined by microbial community composition *and* an appropriate source of energy. In the present study, we compared a system with a specialized root-C-based microbial community to a system inhabited by a community not supported by tree root C. In the 'intact' system, we see clear

seasonal trends, suggesting that FOM degradation, as indicated by actual cellulase/amylase and protease activities, seems to be enhanced in autumn, whereas SOM degradation, as indicated by a higher potential activity of oxidative enzymes, may be enhanced during the summer months. In the girdled system, however, this trend disappeared and lower rates for cellulase and protein degradation, especially in autumn, were found. The possible conclusion that mycorrhizal fungi may be involved in autumn litter degradation, however, still needs to be confirmed by direct measurements.

Overall, our study demonstrated the importance of plant–decomposer interactions for C and N cycling in temperate forests on a seasonal time-scale. Our results showed that extracellular enzymes mediating the decomposition of SOM and litter in a beech forest soil are linked to microbial community composition and exhibit a strong seasonal pattern. Our findings indicate that, through belowground C allocation, trees alter microbial community structure and thus may affect the seasonal pattern of microbial decomposition processes.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Leaf litter fall collected by litter traps in the second sampling year (August–December 2007) in control and girdled plots.

Fig. S2 Microbial community composition of bimonthly samplings on the first two axes of the canonical correspondence analysis.

Table S1 Phospholipid fatty acids of different microbial groups in control, girdled and fertilized soils over a time period of 2 yr.

Table S2 Linear regressions of phospholipid fatty acid biomarkers and soil parameters for control plots only, girdled plots only, and both control and girdled plots together.

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Plants control the seasonal dynamic of microbial N cycling in a beech forest soil by belowground C allocation

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Plants control the seasonal dynamics of microbial N cycling in a beech forest soil by belowground C allocation

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Abstract. Soil microbes in temperate forest ecosystems are able to cycle several hundreds of kilograms of N per hectare per year and are therefore of paramount importance for N retention. Belowground C allocation by trees is an important driver of seasonal microbial dynamics and may thus directly affect N transformation processes over the course of the year. Our study aimed at unraveling plant controls on soil N cycling in a temperate beech forest at a high temporal resolution over a time period of two years, by investigating the effects of tree girdling on microbial N turnover. In both years of the experiment, we discovered (1) a summer N mineralization phase (between July and August) and (2) a winter N immobilization phase (November–February). The summer mineralization phase was characterized by a high N mineralization activity, low microbial N uptake, and a subsequent high N availability in the soil. During the autumn/winter N immobilization phase, gross N mineralization rates were low, and microbial N uptake exceeded microbial N mineralization, which led to high levels of N in the microbial biomass and low N availability in the soil. The observed immobilization phase during the winter may play a crucial role for ecosystem functioning, since it could protect dissolved N that is produced by autumn litter degradation from being lost from the ecosystem during the phase when plants are mostly inactive. The difference between microbial biomass N levels in winter and spring equals 38 kg N/ha and may thus account for almost one-third of the annual plant N demand. Tree girdling strongly affected annual N cycling: the winter N immobilization phase disappeared in girdled plots (microbial N uptake and microbial biomass N were significantly reduced, while the amount of available N in the soil solution was enhanced). This was correlated to a reduced fungal abundance in autumn in girdled plots. By releasing recently fixed photosynthates to the soil, plants may thus actively control the annual microbial N cycle. Tree belowground C allocation increases N accumulation in microorganisms during the winter which may ultimately feed back on plant N availability in the following growing season.

Key words: beech forest; belowground carbon allocation; ectomycorrhiza; N cycle; N retention; plant–soil interactions; seasons; soil microbial community; tree girdling.

Introduction

Terrestrial productivity in temperate forests is among the highest globally, necessitating a fast recycling of N in these latitudes (Huston and Wolverton 2009). Annual uptake of nitrogen into the plant biomass in temperate forests has been found to range between 80 and 150 kg $N \cdot ha^{-1} \cdot yr^{-1}$, which approximately equals annual input of N from aboveground and belowground litterfall (Norby and Iversen 2006, Kreutzer et al. 2009). This high plant N demand is thus met to a high extent (>80%) by ecosystem N recycling, i.e., by microbial mineralization of litter and soil organic matter, whereas external N input by atmospheric N deposition or N_2 fixation only represents a small proportion of the total plant N demand (Schlesinger 1991, Kreutzer et al. 2009). For growth and maintenance of temperate forests, an efficient recycling of N from dead plant material is thus of pivotal importance, especially since (in spite of increasing anthropogenic deposition) N is still a limiting element for the majority of such ecosystems (LeBauer and Treseder 2008, Finzi 2009).

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Microbes have been found to process a lot more N than the plant's annual N demand by depolymerization of soil organic matter and repeated turnover of microbial biomass N over the course of a year (Corre et al. 2007, Schmidt et al. 2007, Kreutzer et al. 2009). These processes lead to a continuous supply of short-term available N in the soil, which is subject to competition between microbes and plants (Schimel and Bennett 2004). The combined N uptake of plant and microbes determines the proportion of N that may potentially be recycled in the system vs. the proportion that is lost by leaching (Gundersen et al. 2006). If NH_4^+ released by microbial decomposition together with external inputs exceed the combined uptake of plants and microbes, a higher proportion of NH_4^+ is transformed to nitrate by microbial nitrifiers (Gundersen et al. 2006, Dise et al. 2009), leading to elevated rates of NO_3^- leaching from the soil with well-known consequences such as soil acidification and eutrophication of ground water and downstream water bodies (Vitousek et al. 1997).

The relative strengths of microbial N release on the one hand, and microbial and plant N uptake on the other hand may, however, vary with seasons leading to different levels of dissolved N that can possibly be lost by leaching at different times of the year. Since leaching of dissolved N from soils occurs presumably at times when both plant and microbial N demand is low (Neff et al. 2003), temporal partitioning of N between plants and soil microbes may be a key mechanism that minimizes N losses from ecosystems. Such a partitioning has been observed in several studies of strongly N-limited ecosystems (Jaeger et al. 1999, Lipson et al. 1999, Schmidt et al. 2007). Schmidt and coworkers (2007) presented a theory of seasonal succession of N cycles in alpine systems: in this concept soil microbes immobilize N in the absence of plant N uptake during autumn and winter, and release N at a time of maximum plant N demand. It was further suggested that fungi in general, which are able to efficiently utilize organic nitrogen from complex plant residues, dominate the microbial community during winter, whereas bacteria, fuelled by root exudates, are more active during summer (Bardgett et al. 2005). Such a concept of seasonal succession of N cycles has, to our knowledge, not yet been tested for temperate forests.

It has been the prevailing opinion until recently that decomposition of plant litter and thus ecosystem N cycling is under the predominant control of soil microbes (Knops et al. 2002). There is now, however, increasing evidence that plants may exert much greater influence on soil N cycling than previously thought (Chapman et al. 2006, Högberg and Read 2006). Recent studies have shown that almost half of the soil respiration in forests is derived from belowground C allocation of recent photosynthates (Högberg et al. 2001, Högberg and Read 2006), and it was further estimated (although with a large uncertainty) that up to 20–30% of the net primary production of temperate and boreal trees is possibly invested to support ectomycorrhizal fungi (Hobbie 2006, Courty et al. 2010). This large input of C to the soil microbial community may in turn strongly affect soil N cycling. A few years ago, Chapman and coworkers (2006) presented a concept of how N cycling may be controlled by different types of plant-decomposer interactions. Fast-growing, N-rich plant species (e.g., some grasses or tropical trees), for example, produce litter which decomposes quickly and exhibit only a loose plant-decomposer coupling. They are mainly associated with arbuscular mycorrhizal fungi, which possess only a low ability to degrade complex compounds (Cornelissen et al. 2001, Read and Perez-Moreno 2003). In this case, litter N is mineralized mainly by free living microbes and N is recycled through the microbial and the labile soil N pool back to plants. More "nitrogen-conservative" plants (such as temperate or boreal trees), on the other hand, produce more recalcitrant, low-nutrient litter and at the same time support high levels of (ecto-)mycorrhizal root colonization (Cornelissen et al. 2001). Ectomycorrhizal fungi are able to degrade complex organic compounds and thus make plant litter N directly accessible to their host-plants (Read and Perez-Moreno 2003). This mechanism short-circuits the mineralization of N through the labile soil N pool and may thereby minimize possible losses of N from the respective ecosystem (Chapman et al. 2006).

These examples demonstrate that plants may pose a strong control on the mechanisms by which N is recycled in the soil. While it is long known that plants may control ecosystem N cycling by producing litter of different substrate qualities, the effect of belowground C allocation on N recycling is less clear. Since temperate forests depend on high N recycling from dead plant biomass, it would be conceivable that the soil microbial community established under the influence of tree C input advances the efficiency of N recycling in the forest soil. While several studies have addressed possible mechanisms regarding how plants may control N cycling in different ecosystems (Chapman et al. 2006) the seasonal aspects of this plant control has received little attention so far. Thus, the aims of our study were (1) to investigate the seasonal microbial N cycle in a temperate beech forest by close monitoring of relevant processes and pools at monthly intervals over a time period of two years, and (2) to elucidate the effect of belowground C allocation and the presence of ectomycorrhizal fungi on the microbial N cycling in a seasonal context.

We used a beech tree girdling experiment to elucidate the role of plant-soil interactions in seasonal microbial N cycling (see Plate 1). Girdling, which interrupts the C flow from the tree canopy to the roots, has led to an approximate 50% reduction of ectomycorrhizal fungi in our study site for a time period of 2–20 months after girdling (Kaiser et al. 2010). We propose that the reduced tree root C input to the soil, which resembles a drawback of plant control on soil processes, will have a

significant effect on the seasonal dynamics of microbial nitrogen cycling in the soil.

As a side effect, girdling has often been found to increase levels of inorganic N in the soil, because reduced C supply for roots may lead to decreased plant N uptake (Edwards and Ross-Todd 1979, Guo et al. 2004). In order to distinguish between the effects that may have been caused by increased inorganic N availability and the effect of reduced belowground C allocation, we additionally established a N fertilization treatment in parallel to the girdling treatment, where we fertilized the soil with NH_4NO_3 in monthly time intervals.

Material and Methods

Study site.—This study was carried out in a temperate beech forest (*Fagus sylvatica*) in Austria, approximately 40 km southwest of Vienna (510 m above sea level). Trees were on average 65 years old. The soil at this site is a dystric cambisol (over flysh), with a pH of 4.5–5.1 ($CaCl_2$) and a C to N ratio of 15.5 (N: 0.48% of dry soil). Despite its proximity to Vienna, the atmospheric N deposition was on average only 12.6 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$ from 2002 to 2004 (Kitzler et al. 2006). Sampling plots were chosen according to a randomized plot design (Kaiser et al. 2010). Briefly, three blocks of approximately 1600 m^2, each of which consisted of more or less homogenous vegetation and soil properties, were selected within a total area of ~5400 m^2. Two control plots (5 × 5 m), two fertilization plots (5 × 5 m) and one girdling area (20 × 20 m) were chosen randomly within each block. Two sampling plots (2.5 × 10 m) were installed within the central 10 × 10 m of each girdling area. Each of them was thus surrounded by a buffer zone of at least 5 m of girdled trees. Each of the three blocks thus contained two replicate plots of each treatment, giving a total of six replicate plots per treatment. Control and fertilization plots were at least 10 m apart from the girdling areas. Within the 20 × 20 m girdling areas, all trees (approximately 30 trees per area) were girdled on 9 May 2006 by removing the bark down to the phloem along a 0.2-m section around the trunk at 1.50 m height (see Plate 1). Understory vegetation was clipped from all plots. Girdling and removal of understory vegetation was repeated in the following spring wherever necessary (some girdled trees produced small amounts of new bark, which did not, however, bypass the girdle). Fertilization plots were fertilized once a month (always after soil sampling) with NH_4NO_3 to obtain a final N fertilization of 50 $kg{\cdot}ha^{-1}{\cdot}yr^{-1}$. The first fertilization took place in May 2006, one month prior to the first sampling (Kaiser et al. 2010). Fertilization was conducted year-round, except in months when the forest floor exhibited a full snow cover, which happened only in the second sampling year (December 2007–February 2008). In this case, the sum of the suspended fertilization doses was applied as an accumulated N input in the first snow-free month (March 2008).

Soil sampling.—Soils from control plots were sampled once a month from June 2006 to June 2008, whereas fertilized and girdled plots were sampled only every two months, except June and July 2007 (which were both sampled), adding up to 24 samplings for controls and 13 samplings for treatments over a time period of two years. Soil was taken from the upper 5 cm of mineral soil (A horizon); from each replicate plot, four subsamples (approximately 250 g soil each) were taken and pooled. In order to avoid sampling of already disturbed soil we used a predetermined sampling scheme. Soil samples were carefully sieved (2 mm), hand-picked from visible roots and kept at 4°C until further processing. All extractions were carried out on fresh soil within 4 days after sampling.

Climatic and abiotic conditions.—Details about the annual course of soil temperature and soil water content in the two sampling years have been presented elsewhere (Kaiser et al. 2010). Briefly, temperature and precipitation patterns were different between the two sampling years such that soil experienced warmer and dryer conditions in the first autumn and winter period (i.e., no snow cover from September 2006 to January 2007) compared to the second autumn/winter. Soil moisture was significantly higher in girdled plots from September to December of both years (by 5–10%), and in spring and summer of the second year (no difference between January and March of both years). Fertilization did not affect soil water content.

Tree vitality.—In the first year of girdling (2006), leaf senescence started approximately three weeks earlier in girdled plots compared to control plots. In the subsequent spring (2007), girdled trees still developed a full flush of leaves. Leaf litter traps, which were installed in May 2007, showed that, although there was no difference in the total amount of leaf litter produced by control and by girdled trees in the second year after girdling, litter fall started notably earlier in girdled plots (34% of total leaf litter fall occurred in girdled plots before the end of September compared to only 13% in control plots) (Kaiser et al. 2010). In spring 2008 (two years after girdling) 40% of girdled trees did not develop new leaves. Fine root biomass in girdled plots did not change 4 months after girdling, but decreased by 45% compared to control plots 14 months after girdling (Kaiser et al. 2010).

DOC and total dissolved N.—Dissolved organic carbon (DOC) and total dissolved N (DN) were measured in water extracts (2 g of fresh soil was extracted with 20 mL laboratory grade water; HgCl was added to a final concentration of 10 μmol/L; extracts were stored at −20°C) and in KCl extracts (2 g of fresh soil was extracted with 20 mL of 1 mol/L KCl; extracts were stored at −20°C) by a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu, Vienna, Austria).

Gross and net ammonification and nitrification rates.—Gross nitrogen transformation rates were assessed using

the pool dilution technique (Myrold and Tiedje 1986): 500 μL of $^{15}NH_4Cl$ (ammonification) or $K^{15}NO_3$ (nitrification) (each 0.25 mmol/L, 10 atom% ^{15}N) were applied to subsamples (2 g) of fresh soil, which were then incubated for 4 h and 24 h (incubation temperature depending on the season: 5°C in winter and 10°C and 15°C in spring/autumn and summer, respectively) and finally extracted with 15 mL of 2 mol/L KCl. For determination of gross ammonification and consumption, NH_3 was diffused from the extracts into acid traps and analyzed for atom-percent excess of ^{15}N by continuous-flow isotope ratio mass spectrometry (IRMS, Delta-PLUS, Thermo Finnigan, Bremen, Germany) using an elemental analyzer as a front-end (EA 1110, CE Instruments, Milano, Italy). For determination of gross nitrification and NO_3^- consumption rates the ^{15}N:^{14}N ratio of NO_3^- was determined by the SPINMAS system (Stange et al. 2007): to achieve this soil extracts were mixed in a reaction vial with 2 mL of acidic $V(III)Cl_3$ solution at 85°C to form NO. The produced NO was transported with helium as a carrier gas (10 mL/min) to the inlet capillary (open split) of a quadrupole mass spectrometer (GAM 400, InProcess Instruments GmbH, Bremen, Germany) where the $\delta^{15}N$ values of the NO were analyzed. Prior the transfer in the quadrupole mass spectrometer H_2O and CO_2 were removed by a cryotrap (−120°C). Rates of gross N mineralization and gross nitrification were calculated according to the following equations (modified from (Bengtson et al. 2006). Net ammonification rates were calculated as the difference between gross ammonification and gross NH_4^+ consumption, i.e., the net change in the NH_4^+ pool over the incubation time (analogue for net nitrification rates, i.e., the net change in the NO_3^- pool over the incubation time),

NH_4^+ transformation rates:

$$\mathrm{gm} = (A_t - A_0)/t \times (\ln[\mathrm{APE}_0/\mathrm{APE}_t]/\ln[A_t/A_0])$$

$$\mathrm{gcm} = \mathrm{gm} - (A_t - A_0)/t$$

$$\mathrm{nm} = (A_t - A_0)/t$$

where gm is gross ammonification, gcm is gross NH_4^+ consumption, nm is net ammonification, A_t is the pool size of NH_4^+-N at time t, A_0 is the initial NH_4^+-N, and APE (atom percent excess) is the at% of ^{15}N in the sample minus at% of ^{15}N of an unlabelled control; and

NO_3^- transformation rates:

$$\mathrm{gn} = (N_t - N_0)/t \times (\ln[\mathrm{APE}_0/\mathrm{APE}_t]/\ln[N_t/N_0])$$

$$\mathrm{gcn} = \mathrm{gn} - (N_t - N_0)/t$$

$$\mathrm{nn} = (N_t - N_0)/t$$

where gn is gross nitrification, gcn is gross NO_3^- consumption, nn is net nitrification, N_t is the NO_3^--N pool after time t, N_0 is the initial NO_3^--N pool.

The rate for which we use the term "gross ammonification rate" in this study (in order to distinguish it from gross nitrification) is sometimes referred to as "gross N mineralization rate" in the literature. Both mean in fact the same rate (measured by the $^{15}NH_4^+$-pool-dilution approach) since ammonification is the first step of N mineralization, and nitrification is thought to be a subset of gross ammonification (organic N is first transformed to NH_4^+ and subsequent to NO_3^-). Gross NH_4^+ consumption includes microbial gross NH_4^+ immobilization *and* losses of NH_4^+ by nitrification and volatilization. We did not, however, subtract nitrification rates from gross NH_4^+ consumption rates to get microbial gross NH_4^+ immobilization rates. Rather, we present all N transformation processes separately, which we think gives the most useful information.

DON, nitrate, and ammonium.—NO_3^- and NH_4^+ were determined from water extracts by chemically suppressed ion-chromatography (HPAEC on a Dionex AS11 column for anions and HPCEC on a Dionex CS16 column for cations [Dionex, Vienna, Austria]; Kaiser et al. 2005). Total dissolved N (DN) was analyzed in water extracts by a TOC/TN analyzer (TOC-V CPH E200V/TNM-1 220V, Shimadzu). Dissolved organic N was calculated by subtracting inorganic from total N.

Microbial biomass C and N.—Microbial biomass C and N was determined by the chloroform-fumigation-extraction method (Amato and Ladd 1988). A subsample (2 g) of fresh soil was fumigated with ethanol-free chloroform in a desiccator for 48 hours and subsequently extracted with 20 mL of 1 mol/L KCl. Microbial C and N was estimated from the difference of organic carbon and total nitrogen measured by a TOC/TN analyzer in KCl extracts of fumigated and unfumigated soils.

Fungal biomass.—Bulk soil DNA was isolated using the FastDNA Spin for Soil Kit (MP Biomedicals, Solon, Ohio, USA) and extracts were quantified photometrically (Nanodrop ND-1000, Nanodrop Technologies, Wilmington, Delaware, USA). SYBR-Green assays for the quantification of fungi were performed in an iCycler iQ5 Multicolor Real Time PCR Detection System (BIO-Rad Laboratories, Hercules, California, USA) using primer pair NSI1 (GATTGAATGGCTTAGTGAGG) (Martin and Rygiewicz 2005)/5,8S (CGCTGCGTTCTT CATCG) (Vilgalys and Hester 1990) as described elsewhere (Inselsbacher et al. 2010). Briefly, 25-μL reactions were composed of 12.5 μL 2× IQ SYBR-Green Supermix (BIO-Rad Laboratories), 0.4 μmol/L of each primer and 0.2 mg/mL BSA. Standards and samples were processed in triplicates. The thermocycler program was set on 95°C for 3 min and 40 cycles of 95°C for 10 s, 60°C (fungi) for 30 s, 72°C for 30 s with data collection at 72°C. Melting curve analysis was done in

order to confirm the specificity of the PCR product. As standard pure culture, genomic DNA from *Cadophora finlandica* PRF15 (Gorfer et al. 2007) was used as reference. To produce the quantitative data for fungal and total DNA (for Fig. 4) three technical repetitions of six biological replicates were analyzed. Mean values from triplicate measurements of each replicate sample were used to produce quantitative data for fungal and total DNA (see Fig. 4).

Results

The seasonal microbial N cycle

The results of our two-year sampling campaign revealed clear seasonal patterns of soil N pools and microbial N processes in a beech forest soil. In particular, we could identify two time periods, namely from July to August and from November to February with distinct trends for specific N parameters.

The pools of water-extractable N in the control soil (dissolved organic N, ammonium, nitrate) peaked in both years during the summer period (July–August), while exhibiting low levels during the autumn and winter period (Fig. 1). Similarly, gross and net ammonification rates (in control plots) peaked during the summer months, and were lowest during the period between November and February (Fig. 2). Net ammonification, which is the difference between gross ammonification (i.e., gross NH_4^+ production) and NH_4^+ consumption, was positive in both years in summer (July/August) and in early autumn (October). This means, that during these time periods, more N was mineralized to NH_4^+, than NH_4^+ was immobilized from the soil solution into the microbial biomass. However, from November until the next spring (again in both years), this pattern turned to the opposite: net ammonification rates were negative, indicating a higher NH_4^+ uptake than release by microbes during this time. Net nitrification showed similar seasonal trends in both years, i.e., high activities in summer, followed by a decline in September and a second peak in autumn before reaching relatively low levels in winter. Despite seasonal variations, net nitrification was almost always positive.

The trend of microbial biomass N was opposite to the trends of extractable N and N mineralization. Microbial N was lowest during the summer period (July–August), increased steeply towards autumn and winter (interrupted only by a short depression between September and October), and then stayed at high levels until spring (again in both sampling years, Fig. 3). The opposing seasonal trends of microbial N on the one hand, and N mineralization and dissolved N pools on the other hand were also reflected in significant negative correlations between microbial N and net mineralization in the control plots ($R^2 = 0.51$, $P < 0.001$) as well as between microbial N and total dissolved N ($R^2 = 0.29$, $P < 0.01$) in both years (Fig. 5). Furthermore, we observed a strong positive relationship between gross mineralization and total dissolved N measured in KCl extracts ($R^2 = 0.78$, $P < 0.001$) and between gross N mineralization and net mineralization ($R^2 = 0.48$, $P < 0.001$). These correlations confirm the seasonal trends we observed for certain N cycling parameters over the course of the year: In the summer phase, microbial biomass N is low while process rates and N availability are high, whereas during the winter period, the situation is precisely the opposite.

Unlike microbial biomass N, microbial biomass C did not show a clear seasonal trend. Microbial C:N ratios used to be higher in the summer compared to the winter, with strong fluctuations, especially in the second sampling year. In both years, we found a steep increase in the microbial C:N ratio between June and August. In the first year, C:N ratio increased from 5 to 12, whereas in the second year from 7 to 22. The increase in the first year was accompanied by an increase in the relative percentage of fungal DNA (Fig. 4). Unfortunately, no DNA data are available for the second year. Total microbial DNA was, similar to microbial biomass N, higher during the winter period compared to the summer period (Fig. 4). Toward December, both fungal and total DNA steeply increased. Fungal DNA decreased in midwinter (February), but total DNA did not, indicating an increasing ratio of bacteria : fungi at that time.

Effects of girdling and fertilization on the seasonal microbial N cycle

Girdling strongly enhanced the amount of ammonium and nitrate in the soil but decreased the amount of DON in the first 18 months after girdling (Fig. 1, Table 1). In the last three months of the sampling period, however, girdling strongly enhanced DON and had a less pronounced effect on ammonium and nitrate. The initial increase of ammonium in girdled plots leveled off at around 250 nmol N/g dry soil in midsummer, whereas nitrate continued to increase during the autumn from 20 nmol up to 250 nmol N/g dry soil, which is more than 10 times higher than the control nitrate level. Fertilization also enhanced nitrate levels but to a lower extent than girdling and showed no clear effect on ammonium or DON.

Girdling significantly decreased gross ammonification over the whole year, but had no overall significant effect on gross NH_4^+ consumption rates (as assessed by factorial ANOVA, Table 1). However, when analyzing only the winter period (November–February) of both years, girdling exhibited a weak significant decrease of gross NH_4^+ consumption rates compared to control plots ($P = 0.096$, Table 1). Girdling affected the seasonal pattern of net ammonification (net NH_4^+ production) rates (Table 1). Although overall net ammonification was decreased on average by girdling in each of the two years, it was significantly enhanced toward positive values during both winter periods in girdled plots ($P = 0.014$, Table 1). This led to a "loss" of the phase of net NH_4^+ immobilization during winter in girdled plots.

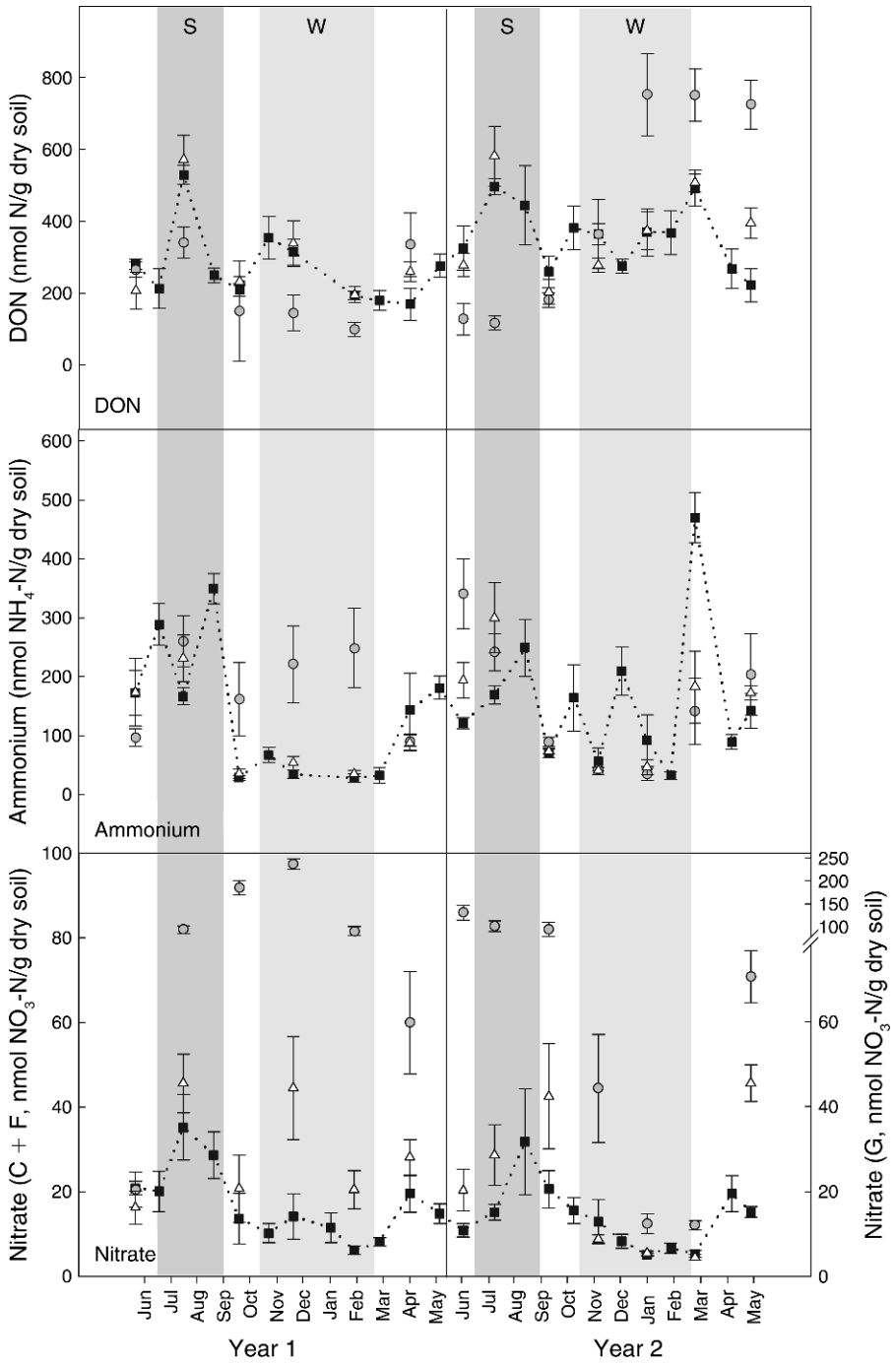

FIG. 1. Dissolved organic nitrogen (DON), ammonium, and nitrate in soil water extracts over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to visualize seasonal trends. Summer (S) and winter (W) periods are highlighted in gray to facilitate identification of seasonal pattern. Note the different scales for nitrate in girdled and control/fertilized plots (C, controls; F, fertilized; G, girdled). All data are plotted on the actual sampling date; ticks indicate the 15th day of each month. Error bars indicate ±SE ($n = 6$).

In contrast to gross ammonification, girdling strongly enhanced gross nitrification rates. It also enhanced gross NO_3^- consumption rates, which resulted in net nitrification rates that were not significantly different between girdled and control plots except for the time period between 0.5 and 1.5 years after girdling. After 1.5 years, the positive effect of the girdling treatment on gross nitrification rates diminished (Fig. 2, Table 1).

The effects of fertilization on gross N transformation rates were generally smaller compared to the effect of

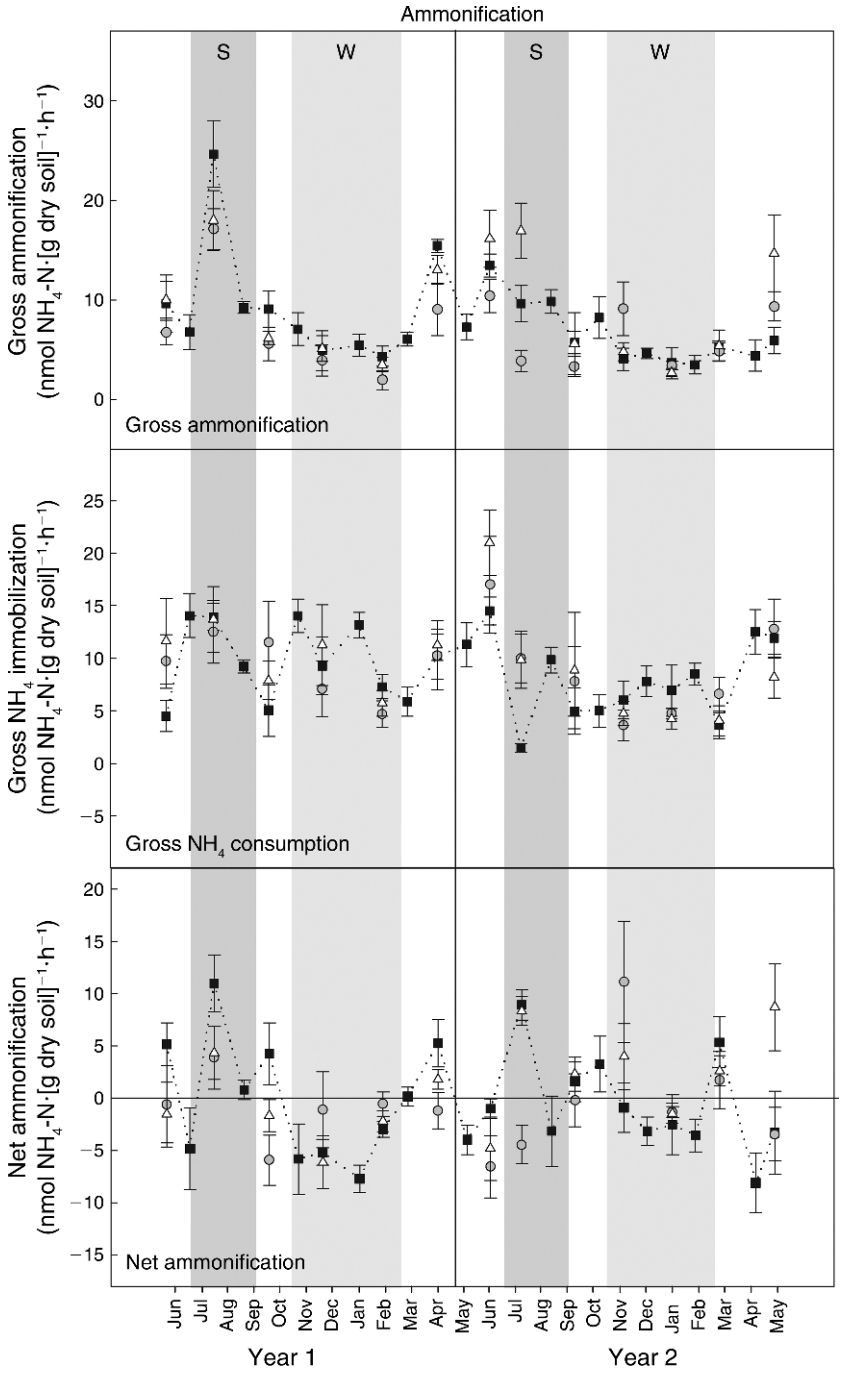

FIG. 2. Nitrogen transformation processes over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to visualize seasonal trends. Summer (S) and winter (W) periods are highlighted in gray. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate ±SE ($n = 6$).

girdling, and varied with sampling dates. Fertilization had a significantly negative effect on net ammonification rates and a positive effect on gross NO_3^- production in the first year. Overall, fertilization had a weak positive effect on nitrification rates (Fig. 2, Table 1).

Tree girdling significantly diminished the build-up of microbial N during the winter period that had been observed for control plots (Fig. 3, Table 1). Midwinter values for microbial N in girdled plots were decreased by 18% and 44% compared to control plots in the first and

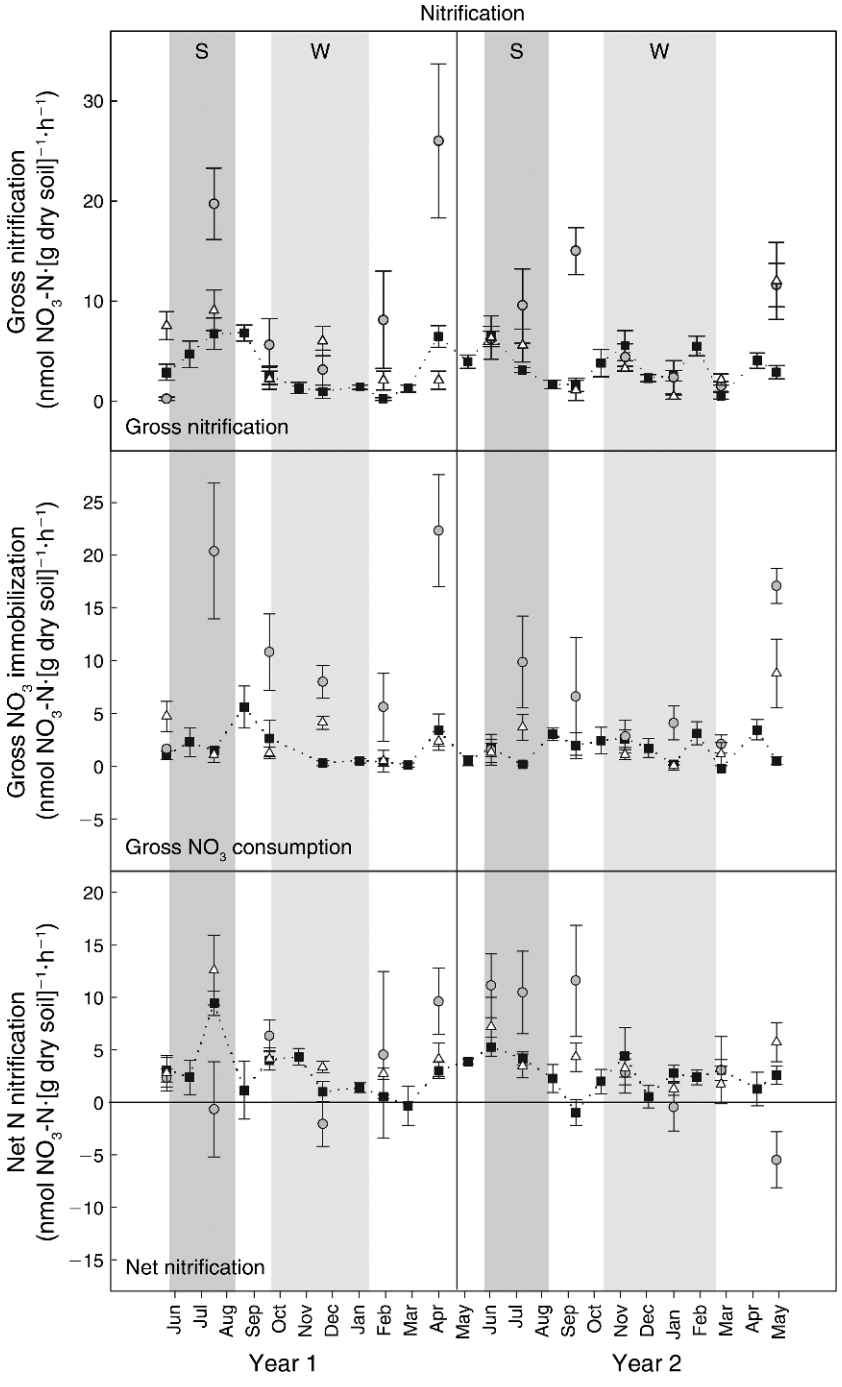

FIG. 2. Continued.

the second sampling year, respectively (Fig. 3). This is consistent with the disappearance of the microbial N immobilization phase (decrease of gross NH_4^+ consumption and increase of net ammonification) during winter in girdled plots (Fig. 2). In contrast, fertilization significantly increased microbial N during autumn and winter (Table 1).

Girdling reduced microbial C only at specific sampling times (e.g., in summer of both years, and in the winter of the second year only). Girdling progressively decreased the abundance of fungal DNA in the first 12 months. The loss of fungal DNA was most pronounced in December (–60%), February (–59%) and April (–69%).

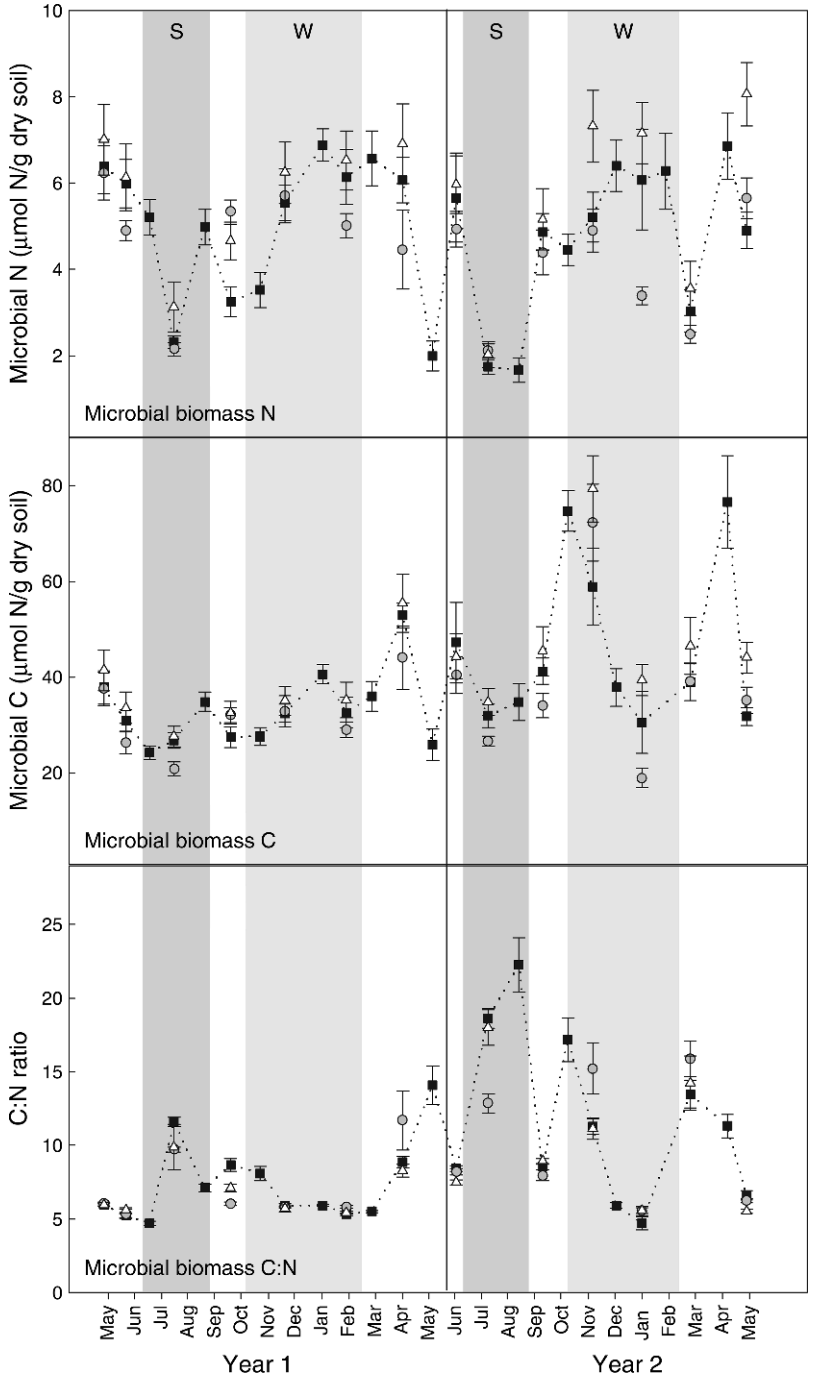

FIG. 3. Microbial carbon and nitrogen over the course of two sampling years (squares, controls; circles, girdled plots; triangles, fertilized plots). Dotted lines connect control values to indicate the seasonal trend. Summer (S) and winter (W) periods are highlighted in gray. All data are plotted on the actual sampling date; ticks are for the 15th day of each month. Error bars indicate ±SE ($n = 6$).

The seasonal trends of microbial N, dissolved N pools and N cycling processes that were present in the control plots, were significantly affected by girdling. In particular the opposing relation between microbial N on the one hand and N pools/processes on the other hand over a year's course disappeared in girdling plots. This was confirmed by the absence of significant negative correlations between these parameters in girdled plots (Fig. 5). By altering the winter increase of microbial N immobilization and reducing the summer peak of N

mineralization, girdling led to a decoupling of the year-round relations between these parameters that was found in control plots.

Discussion

The seasonal dynamics of microbial N cycling may be one of the most important determinants of the ability of ecosystems to retain N (Monson et al. 2006, Schmidt et al. 2007). Although extensive research on the seasonal N cycle has been conducted in alpine and arctic systems, little is known from other ecosystems, in particular about winter processes. Similarly, the effect of tree belowground C allocation and mycorrhizal fungi to the soil microbial N cycle is still not well understood. In this study we provide a set of N pool and process data from a girdling experiment in a temperate beech forest for two full years in order to contribute filling this gap. Our results revealed clear seasonal trends of soil microbial N cycling over the course of a year. In particular, we observed contrasting pictures in summer and in winter. While summer months (July–August) were generally characterized by high N mineralization rates, high dissolved N in the soil solution and low N levels in the microbial biomass, the winter period (Nov–Feb) exhibited the opposite trend: net microbial immobilization of N, low availability of dissolved N in the soil solution, and high levels of N in the microbial biomass. These results point to a strong temporal partitioning of nitrogen between microbes and plants in a beech forest soil. Furthermore, our results revealed a strong plant control over these seasonal patterns: the reduction of belowground C allocation by tree girdling diminished not only the peak summer N mineralization, but also the winter immobilization of N into the microbial biomass, suggesting that plants may actively control the annual forest N re-cycling.

Seasonal patterns of microbial N cycling

The summer peaks of dissolved N and (gross and net) N mineralization and nitrification rates suggest a high availability of N for plants during that time of the year. In a parallel study we observed that extracellular phenoloxidase and peroxidase activities (measured from the same plots) were also at their maximum levels during July and August, while cellulytic enzyme activities were low (Kaiser et al. 2010). This indicates that humified soil organic matter may be the source for increased N mineralization in summer. Concomitantly, we found a steep increase in the microbial C:N ratio between June and August in both years, which was accompanied by an increase in percent of fungal DNA. The wideness of microbial C:N ratios in the summer was a consequence of extraordinarily low levels of N in the microbial biomass whereas levels of microbial C were average. Fungi are known to exhibit more variable C:N ratios compared to bacteria, depending on the C:N ratio of their substrate (Sterner and Elser 2002). It is thus possible that the increase of microbial C:N ratios

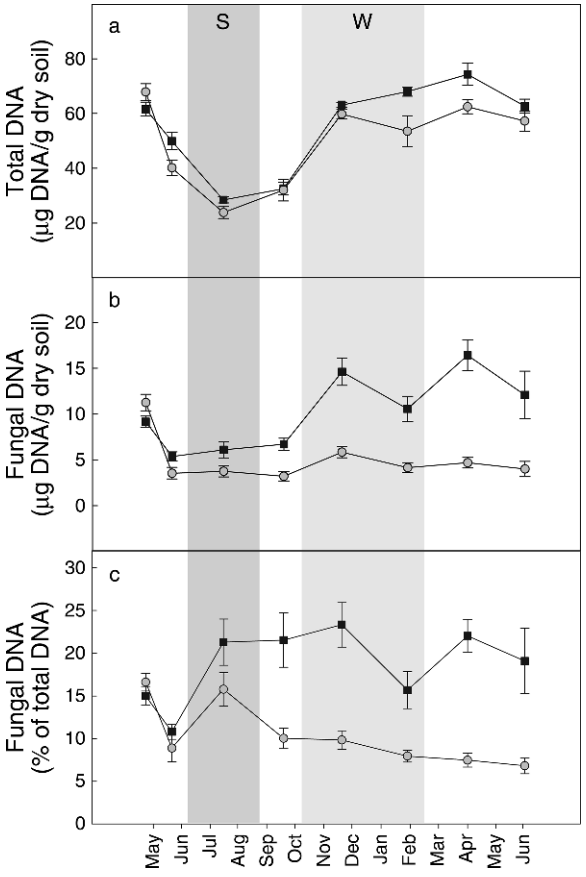

Fig. 4. Fungal DNA and total DNA in soils of a beech forest ecosystem over the course of the first sampling year in girdled and control plots (squares, controls; circles, girdled plots). Summer (S) and winter (W) periods are highlighted in gray. Error bars indicate ±SE ($n = 6$).

reflected the specific situation for microbes in this part of the year (high plant N demand), together with an increase of the fungal population. Although rather high (particularly in year 2), these C:N ratios are still in the range of what was found in other studies on natural communities (Cleveland and Liptzin 2007). Our results of summer soil N trends are consistent with results of a previous study, which reported a peak of available NO_3^- coupled to a substantial decline of microbial biomass N in mid-summer in a beech forest soil (Zechmeister-Boltenstern et al. 2002).

During autumn and winter, when plant N uptake ceased, microbes switched from net N mineralization to net N immobilization which apparently led to an accumulation of N in the microbial biomass over the winter. Again, year-round enzyme measurements at the same study site have shown that actual cellulase and protease activities were at maximum levels during autumn, which points to the degradation of fresh litter during that time being the most important process (Kaiser et al. 2010). Our results suggest that microbes immobilize the N released by enzymatic break-down of

TABLE 1. Effects of girdling, fertilization, and season on N-transformation processes, N pools, and microbial C and N for each sampling year and the winter period of both sampling years, as assessed by ANOVA.

Parameter	Month of sampling	Girdling			Fertilization		
		Difference, G − C	Treatment	Interaction	Difference, F − C	Treatment	Interaction
A) Year 1							
All seasons							
Nitrate	***	1.56	***	***	0.18	**	†
Ammonium	***	0.08	***	**			
DON	***	−0.09	*				
Mineralization							
Gross NH_4 production	***	−4.39	***		−2.27	†	
Gross NH_4 consumption	**						
Net NH_4 production	***	−3.83	**	*	−3.75	**	
Nitrification							
Gross NO_3 production	***	6.81	***	**	1.44	*	**
Gross NO_3 consumption	*	12.36	***				
Net nitrification	†	−0.06		†	1.43	†	
Microbial C	***	−2.97	†		3.02	†	
Microbial N	***	−0.29		**	0.79	*	
Microbial C:N ratio	***	−0.20		***	−0.69	*	
B) Years 1 and 2							
Winter only (Nov–Feb of both years)							
Gross NH_4 consumption		−2.39	†				
Net NH_4 production		5.08	*				
Microbial N		−0.99	*		1.07	*	

Notes: For "all seasons," the effect of sampling month was analyzed for each year by one-way ANOVA using values of all months and all treatments (controls, C; girdled, G; fertilized, F), including months, where only control plots had been sampled ($n = 144$ for year 1 and $n = 156$ for year 2). For the effect of treatments (girdling or fertilization), only data from those months were used (treatment and control), in which the treatment plots were sampled ($n = 72$ for year 1, $n = 84$ for year 2). The effect of each treatment was assessed by a two-way ANOVA, using the respective treatment and sampling month as factors (effect of sampling month not shown; interaction describes interaction between treatment effect and effect of sampling month). For the "winter only" analysis, sampling data from the period of November to February of both years were used ($n = 49$). Units for mean differences between treatment and control plots are μmol NO_3-N/g dm (nitrate), μmol NH_4-N/g dm (ammonium), μmol N/g dm (dissolved organic nitrogen [DON] and microbial N), nmol NH_4-N·[g soil]$^{-1}$·h^{-1} (NH_4 gross production, gross consumption, and net production), nmol NO_3-N·g^{-1}·h^{-1} (NO_3 gross production, gross consumption, and net production), and μmol C/g dm (microbial C), where dm is dry soil mass.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; † $P < 0.1$.

litter (e.g., fine root litter, leaf litter) in autumn and store it at least in part in their biomass over the winter, at a time period when uptake by plants is negligible. This temporal partitioning of N between plants and microbes may be of great importance for ecosystem N retention, especially with regard to the fact, that the major input of fresh organic substrate to the soil in temperate forests occurs in autumn, when plants are thought to be inactive. The most N-rich constituent of plant litter (i.e., proteins) is degraded relatively fast, and the resulting reactive N could thus easily be lost by leaching or volatilization in the absence of microbial immobilization. By storing this N pool at least in part in the microbial biomass over the winter it could be retained in the system and accessed by plants in the following spring. Such temporal partitioning of the N between plants and microbes has been observed before in arctic and alpine systems (Lipson et al. 1999, Schadt et al. 2003, Schmidt et al. 2007). In these studies microbial biomass had been found to peak under late-winter snowpack followed by a significant decline when soils were thawing (Lipson et al. 1999, Schadt et al. 2003), thereby leading to increased N availability for plants at the start of the growing season (Jaeger et al. 1999, Bardgett et al. 2005). Our results strongly suggest that a similar mechanism exists for temperate forests: the spring decline of microbial biomass N (from ~6 to ~2 μmol N/g dry soil) accounts for 38 kg/ha, if calculated on an areal basis, which is as much as one third of the estimated annual N demand of the vegetation of a temperate forest (Kreutzer et al. 2009). This remarkably high amount suggests that N immobilization and storage by soil microbes over the winter may be an essential prerequisite for spring plant growth in a temperate beech forest.

The effect of tree girdling on seasonal patterns of N pools and processes

Although dissolved inorganic and organic N showed the same seasonal trends, they were affected in opposite ways by the girdling treatment, which reduced DON, but strongly enhanced NH_4^+ and NO_3^- levels in the soil.

TABLE 1. Extended.

Month of sampling	Girdling			Fertilization		
	Difference, G − C	Treatment	Interaction	Difference, F − C	Treatment	Interaction
***	0.88	***	***	0.16	***	**
***	−0.07		***	−0.25		***
***	0.00		*			
***	−0.44		**	2.73	*	†
***				1.31		†
***	−1.82		**	1.31		*
***	3.88	***	***	1.12		**
**	5.95	***	**	1.91	*	†
**	1.48		**	0.61		†
***				7.73	**	
***	−0.47		†	1.05	**	
***	−0.03		***			

Root exudates are thought to promote rapid growth of *r*-selected microbes, thereby increasing predation, turnover and subsequent DON availability (Moore et al. 2003). Thus, stopping root exudates by girdling may have led to decreased microbial turnover and decreased DON levels in soil. The same mechanism may have led to reduced ammonification rates in girdling plots. Reduced gross N mineralization rates have also been reported from a girdling experiment in an old-growth spruce forest (Zeller et al. 2008) and gross mineralization rates have been found to be significantly greater in the presence of *Populus* and *Pinus* seedlings compared to non-planted controls (Dijkstra et al. 2009). These findings are consistent with our results, which strongly indicate that belowground C allocation by trees has a positive effect on soil organic matter decomposition and subsequently on gross N mineralization.

In spite of reduced ammonification rates, we observed a strong accumulation of inorganic N (NH_4^+ and NO_3^-) in girdled plots. A possible explanation for this may be that roots, which were cut-off from the C supply by the tree, had to reduce the uptake and assimilation of NH_4^+ and NO_3^- since especially NO_3^- assimilation is an energy-demanding process. The reduced C supply of roots (and thus N uptake capacity) was confirmed by a 45% decline of fine root biomass and a 60% decline of ectomycorrhizal colonization of the remaining vital roots 14 months after girdling (Kaiser et al. 2010). This may have led to the strong accumulation of NH_4^+ in the soil, which, in turn, may have triggered the increase of nitrification and subsequent accumulation of NO_3^- in the soil. The increase of nitrification rates in girdled plots was apparently caused by an increase of bacterial and archaeal nitrifiers in the soil, which had been observed by functional gene analysis of soil samples in the same experiment (Rasche et al. 2010).

A positive effect of girdling on ammonium and nitrate levels in soils has also been observed before in other girdling experiments conducted on various tree species (*Pinus contorta* [Weintraub et al. 2007], *Picea abies* [Zeller et al. 2008], *Fagus sylvatica* [Dannenmann et al. 2009]), although this effect was not always as clear as in our study (Weintraub et al. 2007, Dannenmann et al. 2009). Similarly, reduced DON levels have been observed in some studies (Weintraub et al. 2007), but not in others (Zeller et al. 2008, Dannenmann et al. 2009). These differences may be attributed to different site characteristics but also to different times of girdling and sampling. Our results demonstrate that the response of soil N pools and fluxes to girdling strongly depend on season and on time elapsed since girdling thereby emphasizing the importance of these parameters for interpretation.

The role of mycorrhizal fungi for ecosystem N recycling

Although ammonium and nitrate availability were strongly enhanced in the girdling treatment there was a significantly lower immobilization of N into the microbial biomass over the winter compared to control and fertilization plots. This raises the question, what

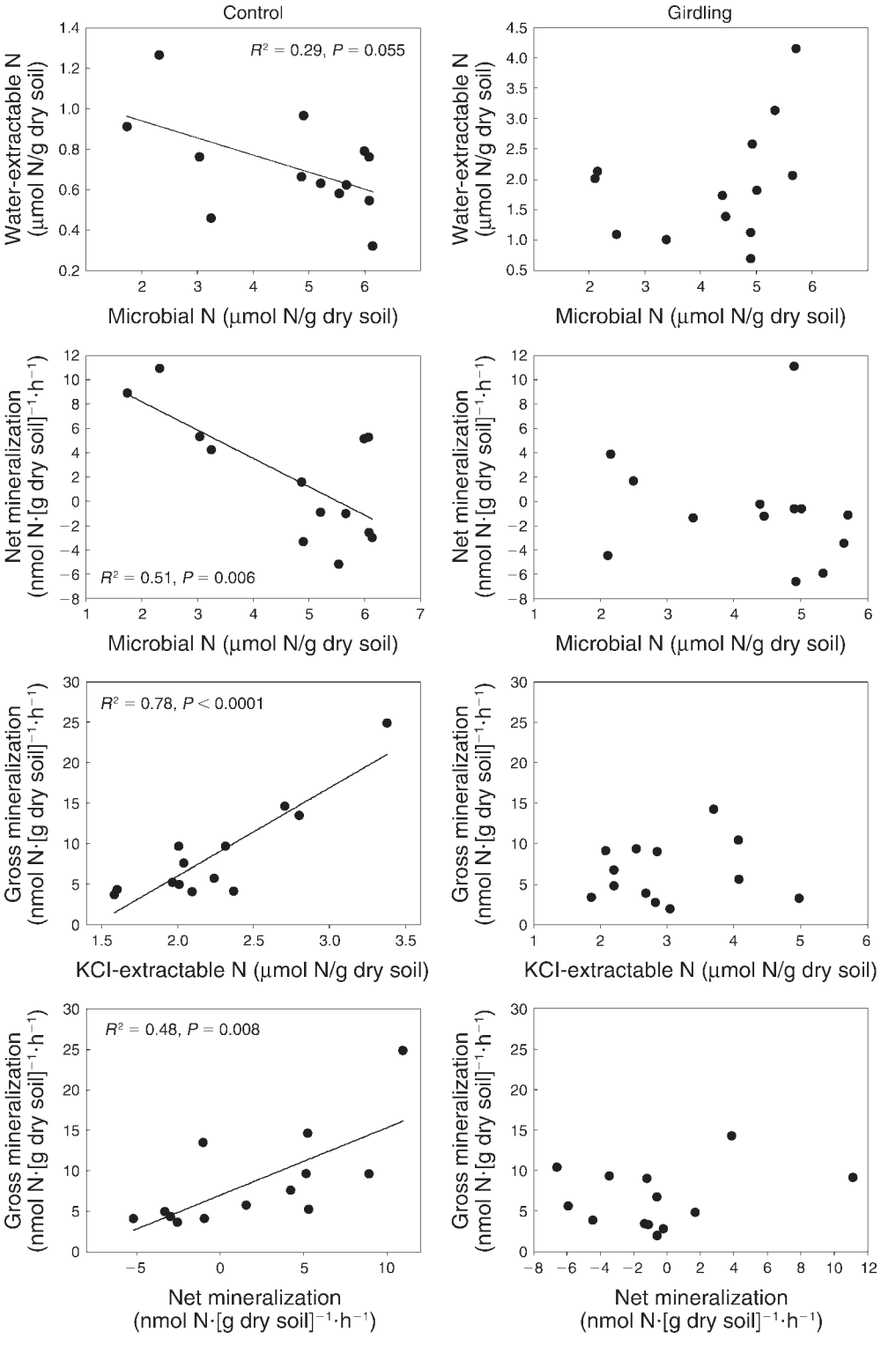

FIG. 5. Correlations between N pools and turnover rates in control and girdled plots. Each point represents the mean value ($n = 6$) of one of 13 sampling dates within two years (bimonthly samplings). The goodness of fit (R^2) and significance of the regression (P value) are given for all cases in which the correlation was significant. Water-extractable N and KCl-extractable N comprise dissolved inorganic nitrogen and dissolved organic nitrogen in water and 1-mol/L KCl extracts, respectively.

PLATE 1. Girdled beech trees (*Fagus sylvatica*) at our study site in Austria in April 2007 (one year after girdling). Photo credit: Franz Hadacek.

actually induced the switch from net mineralization to net immobilization in autumn in control and fertilized plots. An intuitive explanation would be that there is lowered competition with plant roots for N uptake at the end of the vegetation period, which may have enabled microbes to increase immobilization of N. However, our measurements of N mineralization rates were always made in absence of plant roots, which rebuts this argument. Furthermore, if reduced plant N uptake had been the cause of increased microbial N uptake, microbial N immobilization rates would have increased in girdled plots where plant N uptake was reduced and N availability was high. This was, however, not the case. Instead, several other reasons may have led to the switch of microbial N cycling processes in autumn, as for example physiological adaptation of the microbial community to winter conditions, diminishing root C input to the soil and/or a change of the microbial community composition. In particular the stop of root C input to the soil substantially changes the limitation status of the soil microbial community (Fontaine et al. 2003), decreases microbial turnover rates (Moore et al. 2003) and changes microbial community composition (Yarwood et al. 2009, Kaiser et al. 2010). It is noteworthy that, in contrast to girdling, fertilization exhibited a positive effect on microbial winter N storage, especially in the second sampling year. It is thus unlikely that the increased concentrations of inorganic N in the soil of girdled plots may have been responsible for the loss of the wintertime N immobilization phase.

Naturally diminishing root C input towards winter is likely to affect mycorrhizal fungi in the first place. Additionally, girdling strongly decreased mycorrhizal fungi *and* led to the loss of the winter N immobilization phase. It is thus reasonable to consider a possible contribution of mycorrhizal fungi to the increasing microbial N levels towards winter. Mycorrhizal fungi are stronger N sinks than other microorganisms since they are not C limited due to the C supply by their hosts (Högberg et al. 2007, 2008) and due to their ability to translocate C within their mycelia to spots with high N concentration, which enables them to avoid N mineralization even during degradation of N-rich substrate (Boberg et al. 2010). It could thus be, that during summer, N taken up by mycorrhizal fungi is mostly transported to the tree (explaining low N levels in the microbial biomass), whereas during autumn and winter, it could be efficiently immobilized and stored in the fungal hyphae network, which we found to grow to maximum levels during that time (Fig. 4). Our results from the girdling treatment strongly emphasizes this explanation: microbial winter N storage was strongly reduced when tree root C input was stopped by girdling. Since tree girdling changes the C supply compared to control plots only during the photosynthetic active period, this can only be explained by being an effect of the strongly decreased fungal (i.e., mycorrhizal) abundance in girdled plots.

The greatest fluxes of root exudations have been found to occur in the late season (Horwath et al. 1994, Kagawa et al. 2006, Högberg et al. 2010). A recent $^{13}CO_2$ labeling experiment in a boreal forest revealed

that belowground C allocation of plant C was about five times higher in the late season compared to the early season and that the majority of this C was taken up by fungi, as indicated by ^{13}C PLFA-analysis (Högberg et al. 2010). Autumn, however, is also the time of increased N input by litterfall, which has been found to lead to increased cellulase and protease activities (Kaiser et al. 2010). This high simultaneous C and N availability may thus explain the observed increase of total and fungal biomass in autumn. The large input of recent photosynthetates to the soil by trees in the late season may be a plausible mechanism to provide C for mycorrhizal growth, thereby enabling the fungi to accumulate N released by litter degradation. Without the energy from root exudates in autumn, the ability of soil microbes to grow and incorporate N may be limited, as suggested by our girdling plot results. According to our data this limitation strongly affects fungal (especially mycorrhizal) species, emphasizing the important role of mycorrhiza for decomposition and N recycling in temperate forests (Read and Perez-Moreno 2003, Talbot et al. 2008, Wurzburger and Hendrick 2009).

Taken together, our results indicate that temperate (deciduous) trees may actively control the recycling of N originating from the degradation of autumn litter input from one year to the next by supporting a specialized belowground microbial community. Thus, our data expand the recently developed concept that soil N cycling is more controlled by plants and less by microbes than previously thought (Chapman et al. 2006, Högberg and Read 2006, Chapin et al. 2009) for temperate forest ecosystems. Furthermore, our results suggest that the recently observed late season peak in tree root exudates may represent an important function for the plant control on belowground N recycling.

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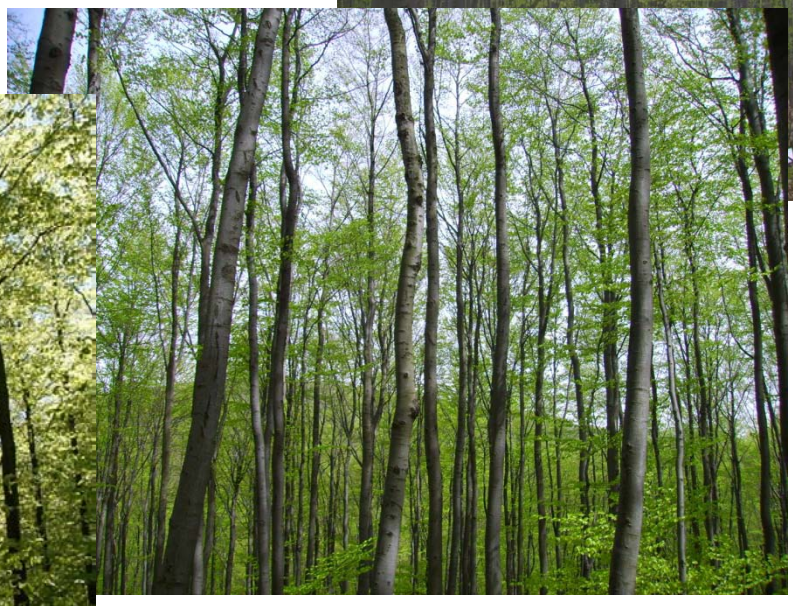
Photos: B. Wild, L. Fuchslueger, F. Hadacek



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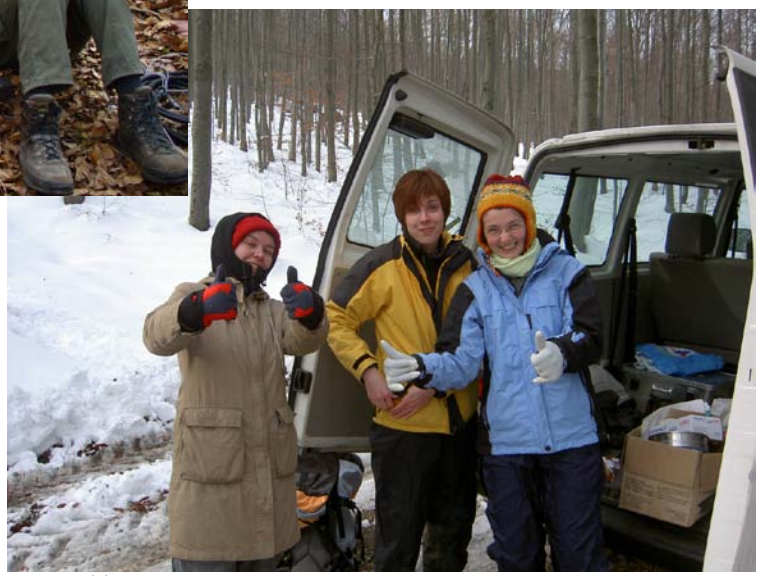

Curriculum vitae

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Born on August 14th 1981 in Linz, Austria

Education

06 / 1999	High school diploma at the Akad. Gymnasium in Linz
1999 - 2006	Degree in biology with focus on botany at the University of Vienna. Diploma thesis on 'Physiological responses of bryophytes to increased N deposition' (Graduation with distinction)
2006 - 2013	PhD-thesis on 'Effects of resource availability and microbial community composition on microbial decomposition processes in beech forest soils' at the Department of Terrestrial Ecosystem Research, Faculty of Life Sciences, University of Vienna

Positions held

2011- 2012 2006 - 2008	Project co-worker at the Department of Terrestrial Ecosystem Research, Faculty of Life Sciences, University of Vienna
2008 - 2010	Project co-worker at the Federal Research and Training Centre for Forests, Natural Hazards and Landscape, Vienna
09/2004, 08/ 2003, 08/ 2002	Trainee at the Federal Research and Training Centre for Forests, Natural Hazards and Landscape, Vienna
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Professional activities

Reviewer for the journals 'Soil biology and biochemistry' and 'Plant and Soil'.

Research interests

(1) Ecosystem processes with focus on plant-soil interactions

(2) Plant ecophysiology; Special interest: bryophytes

Special skills

Experience in chromatography (GC, HPLC, GC-IRMS) and operating software (Chromeleon, Isodat), TOC-analyzer; Experience in working with stable isotopes. Languages: German, English (fluent), French (good), Spanish (moderate).

List of publications

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