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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 initial substrate utilization between distinct microbial communities are due to activities of certain microbial groups. We performed a short-term experiment with beech forest soils characterized by three different microbial communities (winter and summer community, and a community from a tree girdling plot). We incubated these soils with different ^{13}C-labelled substrates with or without inorganic N addition and analyzed microbial substrate utilization by ^{13}C-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. Enhanced N availability strongly increased fungal degradation of cellulose and plant cell walls. Our results hence indicate that fungi, at least under high N supply, are the main decomposers of polymeric C substrates.

Introduction

Due to the high diversity of soil microorganisms it has long been assumed that there is a functional redundancy between distinct microbial communities from different ecosystems and soil types, which implies that different microbial communities have similar functional properties and similar capacities for degradation of a certain substrate (Andren & Balandreau, 1999; Nannipieri *et al.*, 2003; Wertz *et al.*, 2006). 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 phospholipid 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).

There is, however, also evidence of functional redundancy between microbial groups, meaning that bacteria and fungi compete for the same substrates (De Boer *et al.*, 2005; Strickland & Rousk, 2010). 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 degree of functional redundancy or functional differences between different microbial groups regarding decomposition of different substrates is, however, still not completely clear, especially in the context of different nutrient requirements of microbial groups and 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, such that bacteria are more competitive for simple substrates than fungi, and (2) that enhanced inorganic N availability more strongly increases bacterial utilization of C substrates compared to fungal substrate utilization.

We performed an incubation experiment with beech forest soils characterized by three microbial communities. We collected soils in summer and winter as we expected adaptation of the summer community to high availability of labile C and adaptation of the winter community to more recalcitrant substrates (litter). In summer we also collected soil from a tree girdling plot in which belowground carbon allocation had been interrupted which promoted the establishment of a more saprotrophic community. We incubated the different soils with a range of ^{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 for certain groups of microorganisms in order to unravel the fate of decomposed substrates within the soil microbial communities.

Materials and Methods

Soil

The soil for the incubation experiment originated from a 65-year-old beech forest (*Fagus sylvatica*) about 40 km southwest of Vienna (48°07’ N 16°03’ E, 510 m 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) for two weeks until the start of the incubation experiment. Half of the winter soil was transferred to 12°C for equilibration 3 days before the incubation. Soil characteristics (C and N pools, functional microbial communities) of soils collected in winter and in summer from control and from girdled plots 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, N content ~60%, from Sigma) was applied undiluted.

^{13}C-labelled microbial cell walls were prepared as follows: Two bacterial species *(Pectobacterium carotovorum and Verrucomicrobium spinosum*) and one fungal species (*Aspergillus nidulans*) were grown on ^{13}C-glucose (20 atom % ^{13}C). Growth conditions 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) and unlabelled, dried wheat roots were mixed and finely ground and

148 homogenized in a ball mill. The material was then incubated with α-amylase solution to
149 remove starch (Richter et al., 2009) and further extracted repeatedly with
150 methanol/chloroform/water (12:5:3) to remove other labile substances. Plant cell walls
151 contained 0.83% N after removal of labile substances (probably in cell wall proteins).
152
153 *Experimental setup*
154 Soils were sieved (5 mm) and root fragments were removed with forceps. The respective
155 substrate (1 mg substrate g^{-1} soil of glucose and protein (corresponding to ~130 and
156 ~115% of microbial biomass C, respectively) and 4 mg g^{-1} soil of the other substrates)
157 was amended to each soil in a dry form. A subset of the summer soils (from control and
158 girdling plots) amended with either cellulose or plant cell walls, was also amended with
159 inorganic N (3 mg NH_4NO_3 g^{-1} soil). We added N to these treatments in order to test the
160 effects of increased N availability on the degradation of polymeric C substrates (one of
161 them lignin containing), assuming microbial N limitation in summer. For practical reasons
162 (number of samples) we decided to add N to incubations of two substrates only.
163 Incubation of soils (22 g) was performed in a microcosm system with 4 replicates for
164 each substrate and soil (Inselsbacher et al., 2009). For each soil controls without added
165 substrate were prepared (2 x 4 replicates per soil). Microcosms were loosely closed by
166 moist cotton wool and incubated in the dark for 2 days (glucose and protein incubations)
167 or 6 days (microbial cell walls, cellulose and plant cell walls incubations). Incubation
168 times were chosen with the aim to include the peak of $^{13}CO_2$ release following the
169 addition of labile substrates. For incubations of complex substrates, we chose an
170 incubation time of 6 days, which should ensure comparability of the treatments and
171 avoid internal redistribution of ^{13}C within the microbial communities. The anticipated
172 durations of incubations had been determined in a pre-experiment. Incubation
173 temperature was 12°C; additionally soils from the winter community were incubated at
174 4°C. The fate of substrate-C in the winter community did not differ significantly between
175 both incubation temperatures. For better comparability of respiration rates only the
176 results of 12°C winter incubations are presented here. At the end of the incubation
177 period microcosms were destructively harvested for determination of microbial
178 phospholipid fatty acids.
179
180 *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:

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

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 arbuscular mycorrhizal fungi (Olsson, 1999). However, as in beech forest soils ectomycorrhiza is the dominant form of mycorrhiza and moreover, mycorrhizal fungi had probably decayed in our system due

to absence of plant roots, 16:1ω5 is most likely an indicator of Gram-negative bacteria in this soil. PLFAs 18:2ω6,9, 18:2ω3,6,9 and 18:1ω9 were used as fungal markers. The PLFA 20:4ω6,9,12,15 has been proposed as a marker for protozoa (Lechevalier, 1977) or fungi (Stressler *et al.*, 2013).

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

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

Where $APE^{13}C$ means atom % excess ^{13}C in PLFAs and substrate, respectively, and C_{PLFAs} means C content in PLFAs.

Statistics

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

Results

Microbial substrate utilization

In all incubations a peak in substrate respiration shortly after the beginning of the incubation period followed by a decline towards the end of the first day could be observed, most likely because also complex substrates contained a small amount of monomers (Fig. 1). 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 cumulative respiration of labile substrates (Fig. 1, Table 2). While the summer community more intensively respired C from glucose compared to the winter community,

248 a higher respiration of added protein by the winter community was observed. Cumulative
249 respiration of cellulose and plant cell walls was significantly enhanced by the addition of
250 inorganic N ($p<0.001$). At high N availability, soils from girdled plots exhibited higher
251 respiration of complex C substrates than soils from control plots ($p<0.001$).
252 At the end of the incubation period the summer community had incorporated 3-fold as
253 much C from glucose as the winter community (Table 2). Substrate incorporation into
254 PLFAs was significantly enhanced by N addition in incubations of cellulose ($p<0.05$ and
255 $p<0.001$ in control soil and soil from girdled plots, respectively) and slightly increased in
256 incubations of plant cell walls ($p<0.1$ in soils from girdled plots). The microbial
257 community in soil from girdled plots incorporated more C from cellulose than the
258 community in control plots at high N availability ($p<0.05$).
259 Differences in microbial biomass size between soils collected in winter, summer and
260 from girdled plots, however, need to be considered when estimating differences in
261 microbial substrate incorporation and respiration (Microbial biomass C (determined by
262 chloroform-fumigation-method) was higher in summer than in winter, and lowest in soil
263 from girdled plots (37.0, 31.7 and 24.2 μmol g^{-1} DW, respectively)). If values were
264 calculated per unit biomass C, microbial decomposition of complex C substrates
265 (cellulose and plant cell walls) was generally higher in soils from girdled plots than from
266 control plots at both low and high N availability (except for substrate incorporation from
267 plant cell walls; data not shown).
268 The ratio of substrate derived C in PLFAs to respired substrate C was lowest in glucose
269 incubations of the winter community and highest in incubations of microbial cell walls, as
270 well as plant cell walls incubations of the winter community (Table 2). Inorganic N
271 addition to incubations of cellulose significantly increased this ratio ($p<0.001$ and $p<0.05$
272 in soils from control and girdled plots, respectively), while in incubations of plant cell
273 walls the ratio was decreased ($p<0.001$ in soil from control plots) or was not affected by
274 N addition.
275
276 *Distribution of incorporated substrate C within the microbial community*
277 A principal component analysis from relative ^{13}C incorporation into single PLFAs (in
278 percent of ^{13}C in total PLFAs) revealed that the distribution of substrate derived C within
279 the microbial community was mainly determined by the type of added substrate (Fig. 2a;
280 ANOSIM $p = 0.001$, $R = 0.836$). Differences between microbial communities in the
281 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. 2b). 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 into marker PLFAs for different microbial groups (in percent of total incorporated substrate C; Fig. 3). At low N availability the highest proportion of total substrate derived C was generally dectected in marker PLFAs for Gram-negative bacteria, with especially high values being found in incubations of microbial cell walls (~47%), followed by cellulose (33 - 41%) and glucose incubations (~36%). Markers for Gram-positive bacteria incorporated the highest proportion of substrate C in cellulose incubations (between 20 and 27%). Highest substrate incorporation in fungal markers was detected in incubations of glucose (20-23 % of total incorporated C) and plant cell walls (17-20 %). In winter fungal markers incorporated a significantly higher proportion of protein than in summer (19% compared to 15.3%, respectively).

Inorganic N addition not only increased microbial utilization of complex C substrates (Table 2), but also altered the proportional substrate utilization of microbial groups (Fig. 3). 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 (Table 3). In cellulose incubations of soil from girdled plots, however, a dramatic increase in fungal activity by N addition (85-fold increase in ^{13}C incorporation) was accompanied by a significant decline in absolute substrate incorporation of both Gram-positive and Gram-negative bacteria. ^{13}C incorporation of

316 Gram-negative bacteria also tended to decline at high N availability in incubations of
317 plant cell walls.
318 Contrasting to the clear differences in the active microbial community between
319 incubations of different substrates and the strong effects of inorganic N addition on
320 microbial activity, the composition of the total microbial community varied only slightly
321 (Table S1).
322
323 *^{13}C incorporation into specific phospholipid fatty acids*
324 ^{13}C from glucose was detected in nearly all the analyzed PLFAs (Fig. 4a), highest δ^{13}C-
325 values being measured in the fungal marker 18:2ω6 in soil from girdled plots. It has to
326 be kept in mind that δ^{13}C-values depict the ^{13}C incorporation into PLFAs relative to total
327 C in PLFAs (thus normalizing differences in abundances of PLFAs) and not the total ^{13}C-
328 incorporation into PLFAs (given in Fig. S1).
329 In protein incubations (Fig. 4b), substrate ^{13}C was also recovered in most of the fatty
330 acids, similar to the pattern in glucose incubations. Highest δ^{13}C -values were measured
331 in soil collected in winter in the protozoan or fungal marker 20:4ω6, as well as in the fatty
332 acid 16:0 (the precursor molecule for other phospholipid fatty acids (Abraham et al.,
333 1998)), and in the fatty acid i17:1ω8.
334 The pattern of δ^{13}C -values in incubations of microbial cell walls (Fig. 4c), another N-
335 containing substrate, differed considerably from the pattern observed in protein
336 incubations. Only four PLFAs were highly labeled: 20:4ω6, 16:0, and two markers for
337 Gram-negative bacteria, 16:1ω7 and 18:1ω7.
338 δ^{13}C-values in incubations of complex C substrates (cellulose and plant cell walls, Fig.
339 4d and 4e) were much lower than for the first three substrates. In cellulose incubations,
340 the highest label was detected in PLFA 16:1ω5 (Gram-negative bacteria) in soil from
341 girdled plots, while in plant cell wall incubations highest δ^{13}C -values were measured in
342 the fungal markers 18:2ω6 and 18:3ω3. ^{13}C-concentrations in these fungal markers
343 were markedly enhanced by increased inorganic N availability in cellulose incubations
344 (in both soils from control and girdled plots) and in plant cell wall incubations in soils
345 from girdled plots, while the high ^{13}C-incorporation from cellulose in PLFA 16:1ω5 nearly
346 dropped to zero at high N availability. According to changes in δ^{13}C -values of marker
347 PLFAs for Gram-positive bacteria by inorganic N addition, two different types of Gram-
348 positive marker PLFAs (probably characterizing different genera or families) could be
349 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, $\delta^{13}C$ -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 (probably reflecting adaptation of the summer community to high availability of labile C supplied by plants via belowground C allocation) (Table 2), but the distribution of substrate C within the microbial community was similar in both seasons (Fig.2, 3). This is in line with results from other studies which demonstrated that labile C substrates are equally metabolized by a large range of microbial species (Waldrop & Firestone, 2004; Paterson *et al.*, 2011), predominantly by Gram-negative bacteria and fungi (Treonis *et al.*, 2004; Bird *et al.*, 2011). The low ratio of substrate C in PLFAs over respired substrate C in incubations of glucose, which indicates microbial N limitation, may result from substrate C being stored in neutral lipids (of fungi) instead of being used for growth, as reported in other studies (Baath, 2003; Rinnan & Baath, 2009). Functional differences between summer and winter communities in utilization of another labile substrate, protein (Table 2) seemed to be linked to fungal activity, as indicated by higher fungal substrate incorporation in winter soil (Fig. 3). This contrasts results from another study (Rinnan & Baath, 2009) reporting that glycine was predominantly utilized by bacteria, while fungal utilization of this substrate was low. Unlike protein, microbial cell walls were predominantly utilized by Gram-negative bacteria (which also showed a very high substrate use efficiency for this substrate), suggesting that they either have a unique set of enzymes for chitin and/or peptidoglycan decomposition or that they are especially efficient in taking up the products of decomposition (i.e., are efficient cheaters).

Degradation of complex C substrates was higher in soils from girdled plots than from control plots (Table 2), possibly indicating microbial adaptation to high amounts of dead fine root biomass in girdled plots three years after girdling. In cellulose incubations, as

384 well as cellulose and plant cell walls incubations with inorganic N additions, microbial
385 communities from girdled and control plots also exhibited a differing pattern of substrate
386 incorporation of microbial groups (Fig. 2, 3), probably reflecting enhanced activity of
387 saprotrophic microbes specialized on degradation of polymeric C substrates in soil from
388 girdled plots. Functional differences between distinct microbial communities in the
389 degradation of recalcitrant substrates, such as plant litter, which were linked to
390 differences in the distribution of substrates within the microbial communities, have
391 already been described previously (Waldrop & Firestone, 2004; Paterson *et al.*, 2011).
392 In most studies, fungi were reported to be the main decomposers of lignocellulose (De
393 Boer *et al.*, 2005; Paterson *et al.*, 2008). This has, to a large extent, been confirmed by
394 our findings. Our results revealed, however, that the composition and activity of the
395 microbial community involved in the decomposition of polymeric C substrates was
396 strongly dependent on nutrient availability. At low N availability a large proportion of
397 substrate C from cellulose was incorporated in bacterial markers, especially in soil from
398 girdled plots, while fungal incorporation of this substrate into PLFAs was low (Fig. 3, 4;
399 Table 3). The capacity of different bacterial taxa for degradation of cellulose has already
400 been reported in other studies (De Boer *et al.*, 2005; Vargas-Garcia *et al.*, 2007;
401 Goldfarb *et al.*, 2011). Bacterial utilization of cellulose, however, should be interpreted
402 with caution. First, bacterial cellulose degradation is probably facilitated by the finely
403 ground form of cellulose applied in our experiment, which makes it more easily
404 accessible for bacteria. Second, in cellulose incubations without added N, most
405 substrate C was respired in the initial phase of the incubation, which suggests that
406 bacteria mainly utilized labile compounds contained in the cellulose (e.g., oligomeric
407 sugars). Actual cellulose degradation by bacteria is hence questionable. Longer
408 incubation times would have probably been needed in order to assess bacterial
409 participation in cellulose decomposition.
410 Contrary to bacteria, fungi more intensively utilized plant cell walls than cellulose (Fig. 3,
411 4), which is probably due to the lignin content of plant cell walls, as well as N contained
412 in cell wall proteins.
413 If inorganic N was added to cellulose and plant cell walls, fungal decomposition activity
414 of polymeric C substrates strongly increased (Fig. 3, 4; Table 3). N addition also
415 significantly enhanced the ratio of incorporated substrate C over respired substrate C
416 (Table 2) in incubations of cellulose, but not of plant cell walls, which suggests strong N
417 limitation of fungi in cellulose incubations without inorganic N. The low amount of

418 substrate C in fungal marker PLFAs in cellulose incubations without added N may thus
419 underestimate total fungal substrate incorporation, as substrate C from cellulose may be
420 used for synthesis of storage compounds instead of growth at low N availability (Baath,
421 2003). This also implies a low production of extracellular enzymes under conditions of N
422 limitation. Addition of inorganic N alleviated fungal N limitation, resulting in strongly
423 enhanced decomposition rates of complex C substrates, increased respiration rates and
424 increased fungal growth, i.e. substrate incorporation in PLFAs. This positive effect of N
425 addition on fungal activity is remarkable, since fungi are generally assumed to have
426 lower N demands than bacteria, due to their higher biomass C:N ratio (Keiblinger *et al.*,
427 2010; Strickland & Rousk, 2010). Stimulation of fungal growth or activity by enhanced N
428 availability, however, has also been found in other studies (Rousk & Baath, 2007;
429 Boberg *et al.*, 2008; Meidute *et al.*, 2008; Fontaine *et al.*, 2011), which applied lower N
430 loads than in our study. The often observed fungal dominance in the decomposition of
431 N-poor plant litter hence seems to be to a great extent due to the hyphal growth form,
432 which enables fungi, apart from the penetration of plant cell walls, to import N from N-
433 rich soil horizons (e.g. from degradation of SOM) (Frey *et al.*, 2003; Strickland & Rousk,
434 2010; Fontaine *et al.*, 2011).
435 Bacterial incorporation of polymeric C substrates, on the other hand, tended to be
436 reduced by N addition (Fig. 3, 4; Table 3). Negative responses of Gram-negative
437 bacteria to N fertilization, especially in the abundance of the marker PLFA 16:1ω5, have
438 also been reported in a previous field study (Weand et al., 2010).
439 Our results on microbial utilization of polymeric C substrates corroborated in part
440 findings by others. Rousk *et al.* (2008) observed that fungi and bacteria may compete for
441 the same complex substrates, indicating functional redundancy between microbial
442 groups with respect to decomposition processes. In our short-term study, however,
443 mainly fungi were responsible for decomposition of cellulose and plant cell walls, but
444 bacterial decomposition of such substrates at longer incubation times could have been
445 possible. Changes in N availability strongly influenced competitive abilities of microbial
446 groups, fungi outcompeting bacteria at high N availability. In addition, at sufficient N
447 supply the overall decomposition rates increased substantially, indicating that fungi are
448 probably more efficient decomposers of complex C substrates compared to bacteria.
449
450 In summary, our results revealed that differences in utilization of a labile C substrate
451 (glucose) between microbial communities (summer and winter community) were not due

452 to activities of a certain microbial group, but reflected differences in substrate utilization
453 by a large part of the microbial community. This utilization pattern for labile substrates
454 contradicts our initial hypothesis that distinct microbial communities differ in utilization of
455 organic substrates and in partitioning of substrate derived C within the microbial
456 community. Higher degradation of polymeric C substrates (cellulose and plant cell walls)
457 in soils from girdled plots than from control plots, however, was related to an altered
458 composition of the active microbial community in girdled plots.
459 Decomposition of complex C substrates was markedly increased by enhanced inorganic
460 N availability, with strongly divergent effects on different microbial groups. Contrary to
461 our hypothesis, N addition strongly enhanced fungal decomposition of cellulose and
462 plant cell walls, while bacterial utilization of these substrates was not significantly
463 changed or even reduced. Although the results from this short-term experiment cannot
464 be extrapolated to the field, our results indicate that fungi, at least under high N supply,
465 are the main decomposers of polymeric C substrates.
466
467
468 **Acknowledgements**
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470

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590
591

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

595

	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'. Data based on Koranda *et al.* (2013).

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

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

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

Legends to figures

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

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

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

Figure 4
^{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).

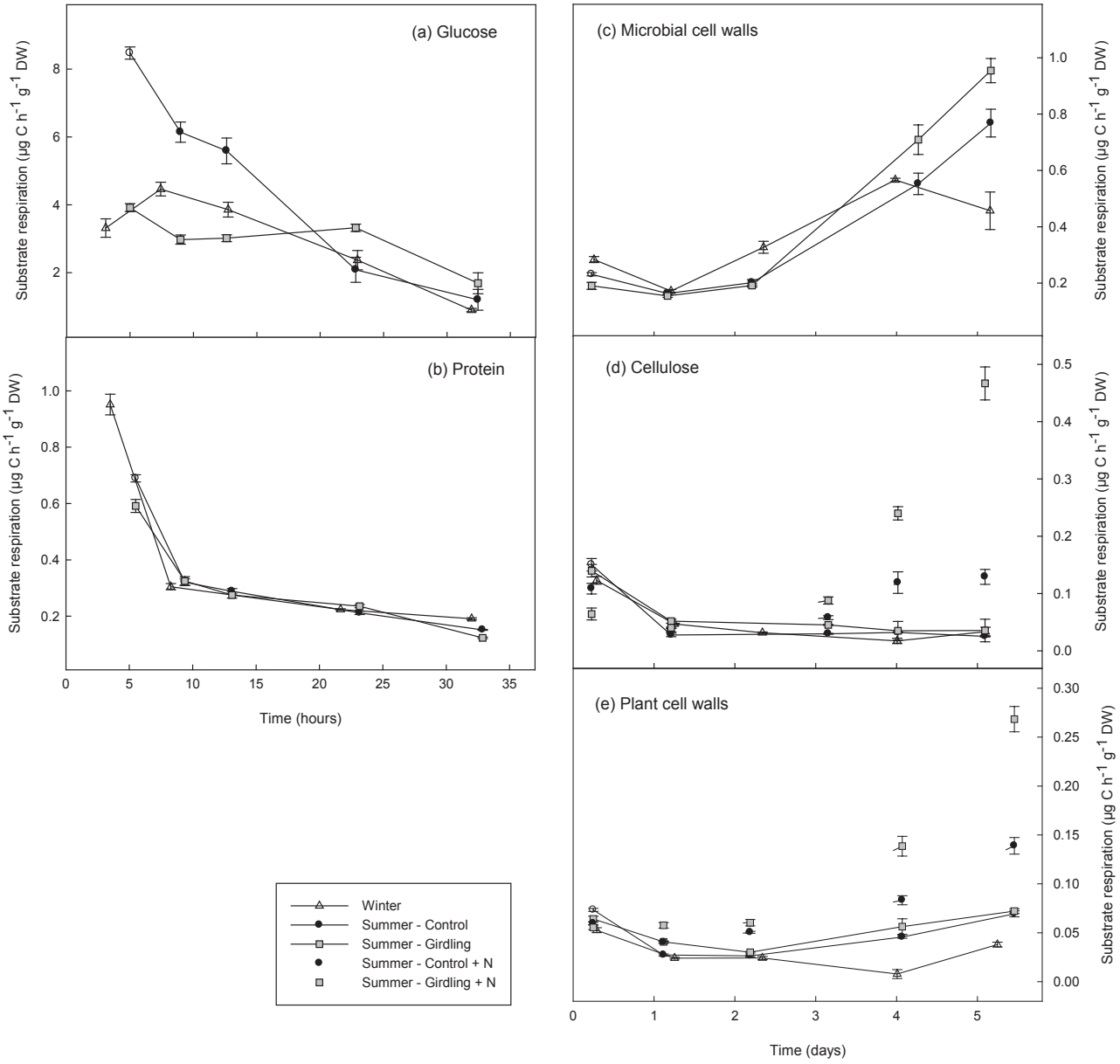
(a) Glucose
(b) Protein
(c) Microbial cell walls
(d) Cellulose
(e) Plant cell walls
Substrate respiration (µg C h-1 g-1 DW)
Time (hours)
Time (days)
Winter
Summer - Control
Summer - Girdling
Summer - Control + N
Summer - Girdling + N

Fig. 1

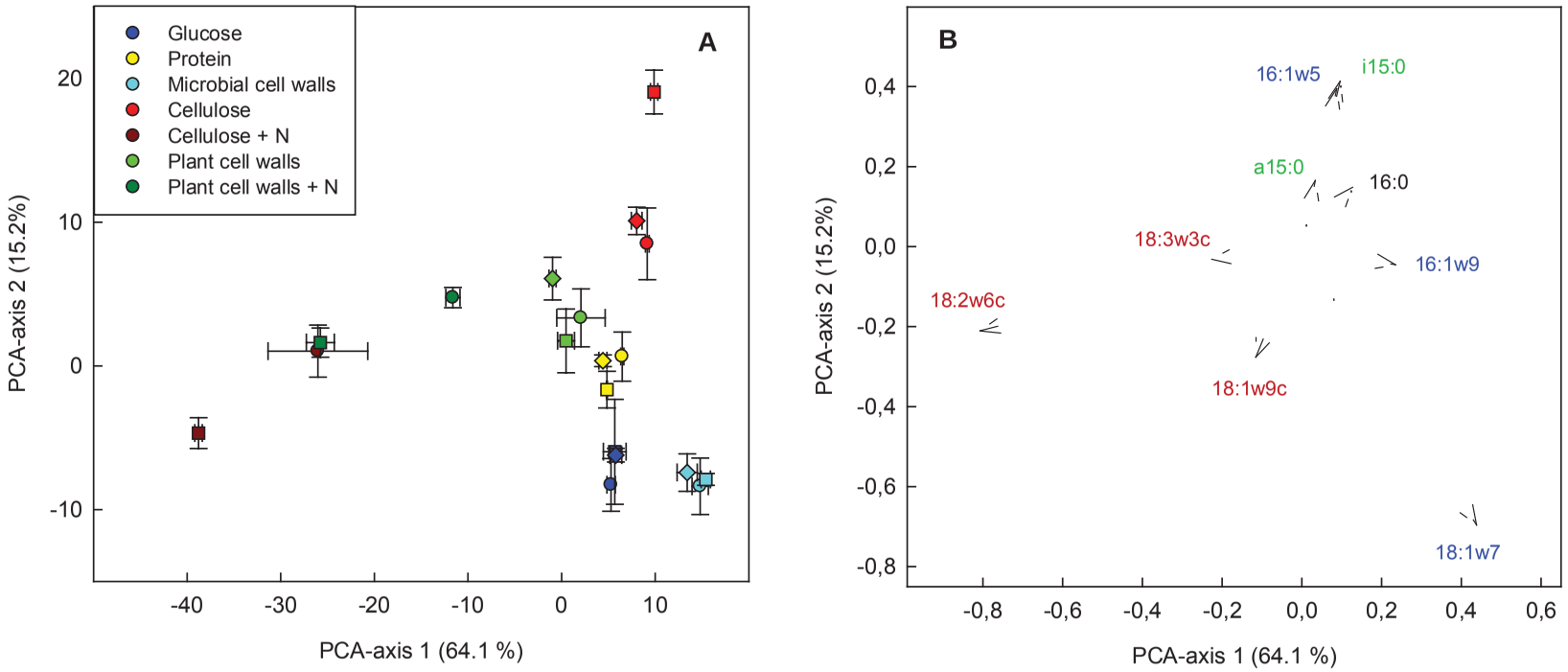
A
Glucose
Protein
Microbial cell walls
Cellulose
Cellulose + N
Plant cell walls
Plant cell walls + N
PCA-axis 2 (15.2%)
PCA-axis 1 (64.1 %)
20
10
0
-10
-40
-30
-20
-10
0
10
B
16:1w5
i15:0
a15:0
16:0
18:3w3c
16:1w9
18:2w6c
18:1w9c
18:1w7
PCA-axis 2 (15.2%)
PCA-axis 1 (64.1 %)
0,4
0,2
0,0
-0,2
-0,4
-0,6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

Fig. 2

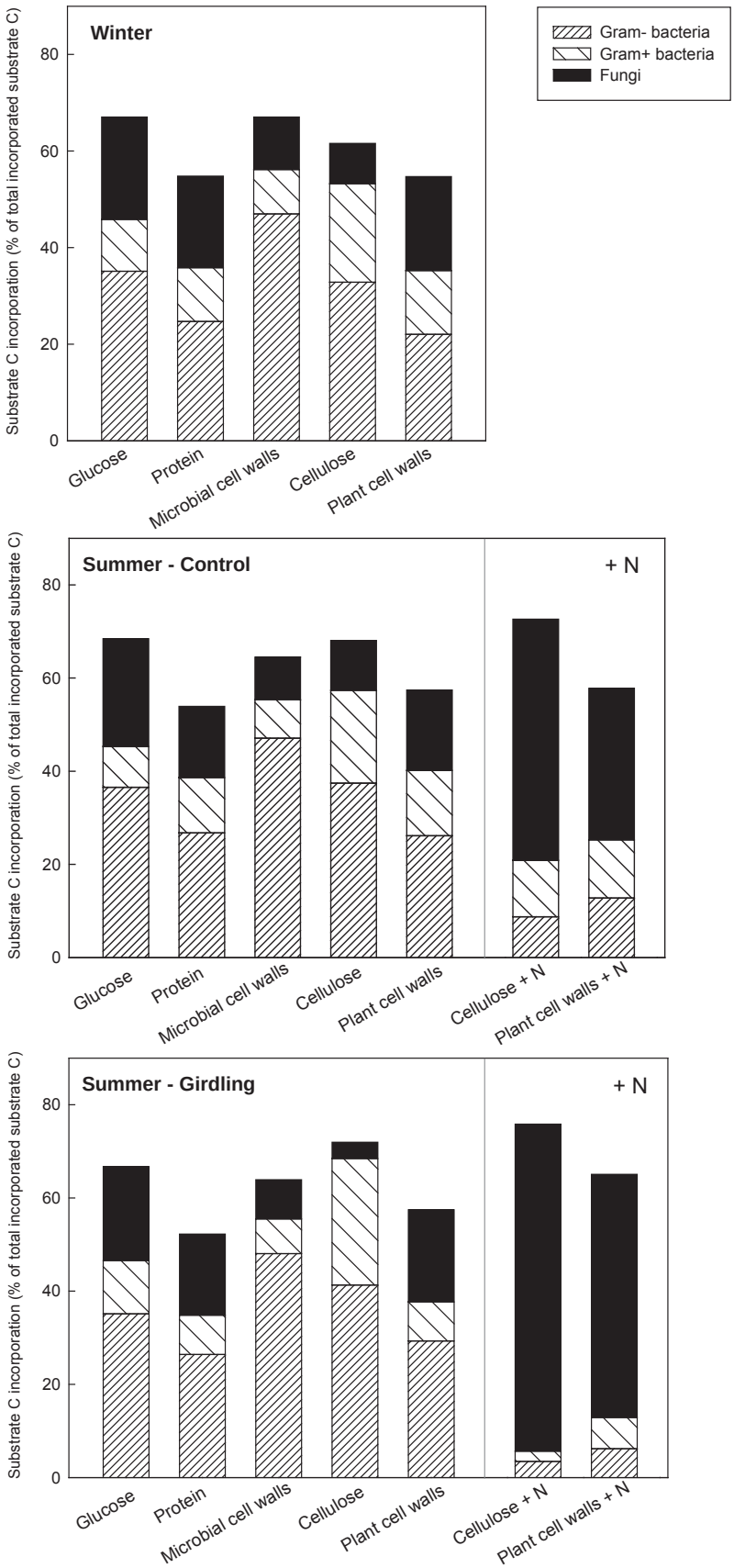
Winter
Gram- bacteria
Gram+ bacteria
Fungi
Substrate C incorporation (% of total incorporated substrate C)
0
20
40
60
80
Glucose
Protein
Microbial cell walls
Cellulose
Plant cell walls
Summer - Control
+ N
Cellulose + N
Plant cell walls + N
Summer - Girdling

Fig. 3

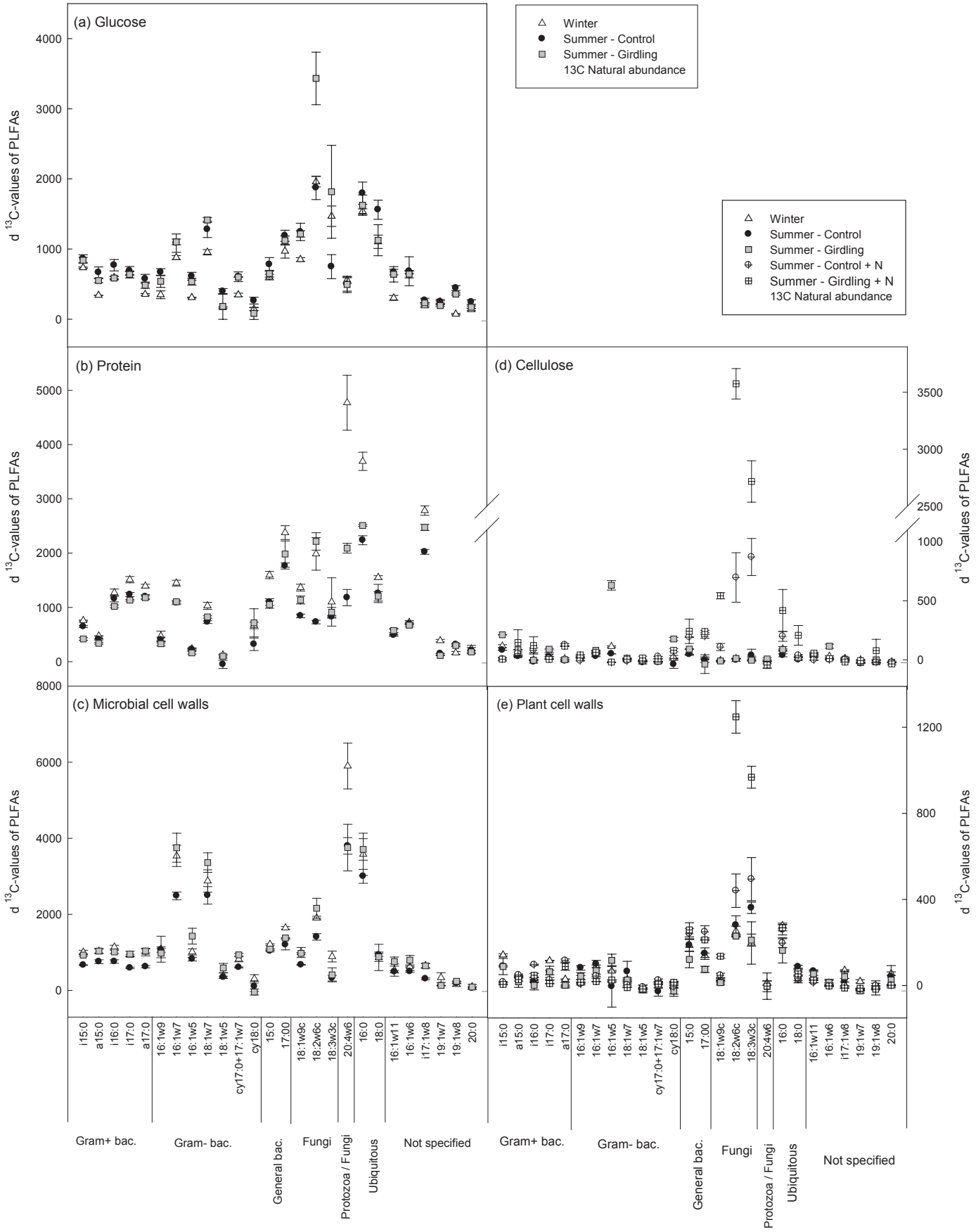
(a) Glucose
(b) Protein
(c) Microbial cell walls
(d) Cellulose
(e) Plant cell walls
Winter
Summer - Control
Summer - Girdling
Summer - Control + N
Summer - Girdling + N
13C Natural abundance
d 13C-values of PLFAs
i15:0
a15:0
i16:0
i17:0
a17:0
16:1w9
16:1w7
16:1w5
18:1w7
18:1w5
cy17:0+17:1w7
cy18:0
15:0
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 / Fungi
Ubiquitous
Not specified

Fig. 4

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	
Glucose	51.5	(2.0)	17.8	(2.2)	32.2	(0.5)	12.5	(0.4)
Protein	51.0	(1.9)	18.6	(1.4)	30.7	(0.5)	14.3	(1.1)
Microbial cell walls	50.4	(0.1)	17.8	(0.7)	30.9	(0.7)	12.9	(0.2)
Cellulose	49.7	(1.0)	17.2	(1.2)	30.5	(0.9)	14.4	(0.7)
Plant cell walls	49.0	(2.5)	16.3	(2.6)	31.2	(1.2)	15.4	(1.2)
Summer - Control								
Glucose	48.6	(1.8)	14.3	(2.7)	32.7	(1.0)	15.0	(1.1)
Protein	49.4	(1.4)	16.1	(2.7)	31.4	(1.4)	15.3	(1.3)
Microbial cell walls	50.8	(1.8)	19.0	(2.6)	30.1	(0.9)	12.6	(0.5)
Cellulose	46.9	(1.8)	13.9	(4.0)	31.3	(2.4)	15.2	(1.8)
Cellulose +N	49.4	(1.2)	21.5	(0.3)	26.1	(0.8)	14.4	(0.6)
Plant cell walls	52.0	(0.3)	20.8	(0.4)	29.1	(0.3)	12.4	(0.4)
Plant cell walls +N	45.9	(2.7)	18.1	(1.9)	26.1	(0.8)	13.6	(0.3)
Summer - Girdling								
Glucose	49.2	(2.2)	19.7	(5.1)	27.9	(2.9)	10.1	(1.7)
Protein	45.5	(0.3)	13.5	(0.1)	30.1	(0.3)	11.7	(0.5)
Microbial cell walls	47.3	(0.1)	16.5	(0.7)	29.2	(0.7)	11.3	(0.6)
Cellulose	49.1	(1.9)	19.8	(2.7)	27.7	(0.9)	11.1	(0.8)
Cellulose +N	42.6	(1.4)	14.4	(2.9)	25.3	(0.2)	14.6	(0.2)
Plant cell walls	44.0	(1.0)	11.5	(1.5)	30.7	(0.5)	13.3	(0.3)
Plant cell walls +N	46.2	(2.6)	19.5	(4.0)	25.0	(1.6)	12.3	(1.3)

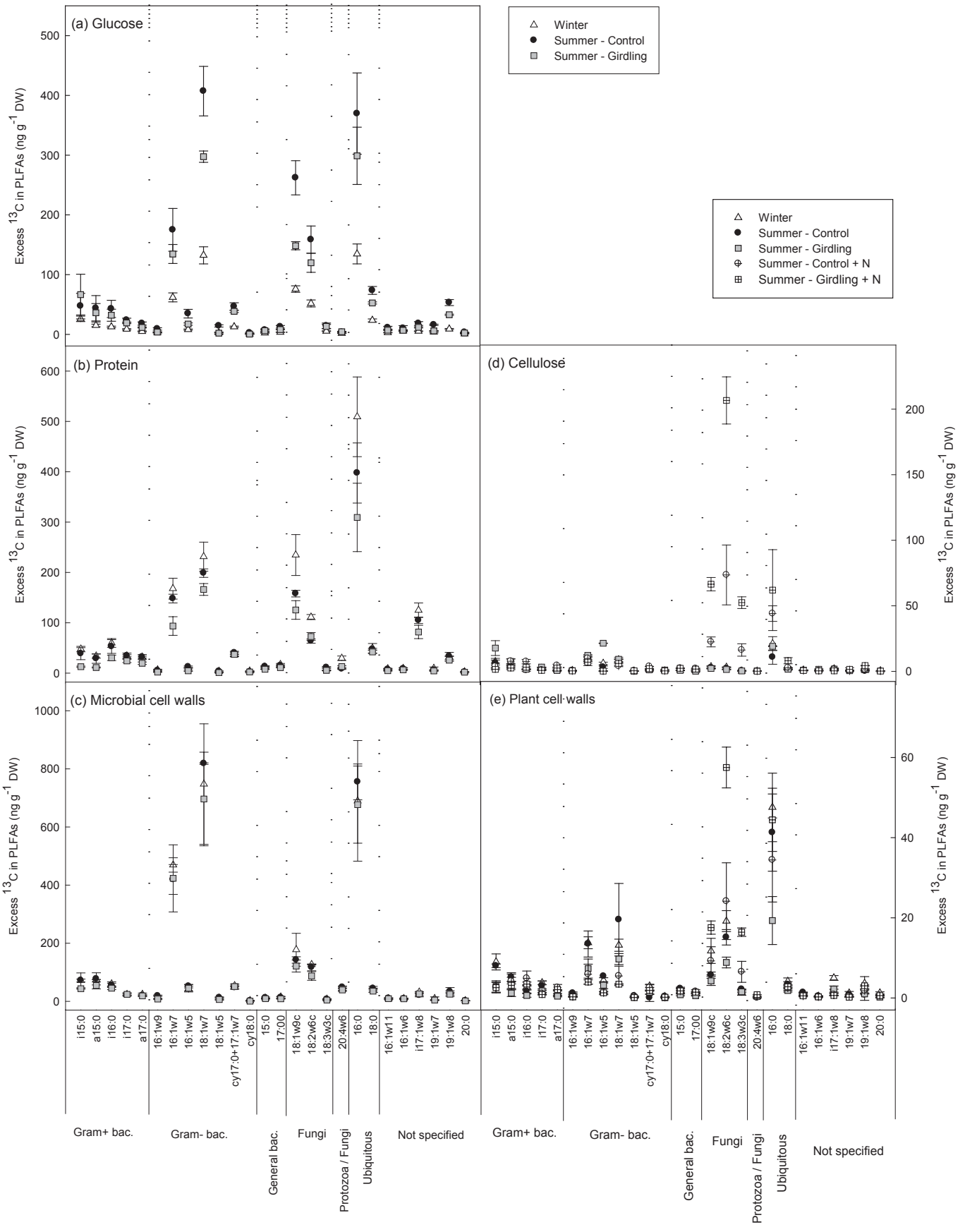
(a) Glucose
(b) Protein
(c) Microbial cell walls
(d) Cellulose
(e) Plant cell walls
Winter
Summer - Control
Summer - Girdling
Summer - Control + N
Summer - Girdling + N
Excess 13C in PLFAs (ng g-1 DW)
i15:0
a15:0
i16:0
i17:0
a17:0
16:1w9
16:1w7
16:1w5
18:1w7
18:1w5
cy17:0+17:1w7
cy18:0
15:0
17:00
18:1w9c
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 / Fungi
Ubiquitous
Not specified

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