



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

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

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

3

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21

22

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24

25 Running title: Fungal and bacterial utilization of organic substrates

26

27 **Abstract**

28

29 There is growing evidence of a direct relationship between microbial community
30 composition and function, which implies that distinct microbial communities vary in their
31 functional properties. This study aimed at elucidating whether differences in initial
32 substrate utilization between distinct microbial communities are due to activities of
33 certain microbial groups. We performed a short-term experiment with beech forest soils
34 characterized by three different microbial communities (winter and summer community,
35 and a community from a tree girdling plot). We incubated these soils with different ¹³C-
36 labelled substrates with or without inorganic N addition and analyzed microbial substrate
37 utilization by ¹³C-PLFA analysis. Our results revealed that the fate of labile C (glucose)
38 was similar in the three microbial communities, despite differences in absolute substrate
39 incorporation between summer and winter community. The active microbial community
40 involved in degradation of complex C substrates (cellulose, plant cell walls), however,
41 differed between girdling and control plots and was strongly affected by inorganic N
42 addition. Enhanced N availability strongly increased fungal degradation of cellulose and
43 plant cell walls. Our results hence indicate that fungi, at least under high N supply, are
44 the main decomposers of polymeric C substrates.

45

46 **Introduction**

47

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

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

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

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

74 There is, however, also evidence of functional redundancy between microbial groups,
75 meaning that bacteria and fungi compete for the same substrates (De Boer *et al.*, 2005;
76 Strickland & Rousk, 2010). Added labile C substrates were found to be equally utilized
77 by a broad range of community members (Waldrop & Firestone, 2004; Paterson *et al.*,
78 2011). There is also evidence of competition between bacteria and fungi for complex
79 substrates (Rousk *et al.*, 2008; Strickland & Rousk, 2010), which is in line with

80 observations that certain bacterial species have the physiological capacities for
81 degradation of polymeric C substrates and even lignified substrates (Perestelo *et al.*,
82 1996; Vargas-Garcia *et al.*, 2007). The degree of functional redundancy or functional
83 differences between different microbial groups regarding decomposition of different
84 substrates is, however, still not completely clear, especially in the context of different
85 nutrient requirements of microbial groups and resulting competitive advantages.
86 We have recently reported that distinct microbial communities differed in their functional
87 properties (Koranda *et al.*, 2013). Here we focus on the question whether differences in
88 functional properties of microbial communities are related to activities of certain microbial
89 groups. We hypothesized (1) that distinct microbial communities differ in utilization of
90 organic substrates and in partitioning of substrate derived C within the microbial
91 community, such that bacteria are more competitive for simple substrates than fungi,
92 and (2) that enhanced inorganic N availability more strongly increases bacterial
93 utilization of C substrates compared to fungal substrate utilization.
94 We performed an incubation experiment with beech forest soils characterized by three
95 microbial communities. We collected soils in summer and winter as we expected
96 adaptation of the summer community to high availability of labile C and adaptation of the
97 winter community to more recalcitrant substrates (litter). In summer we also collected
98 soil from a tree girdling plot in which belowground carbon allocation had been interrupted
99 which promoted the establishment of a more saprotrophic community. We incubated the
100 different soils with a range of ¹³C-labeled substrates (glucose, protein, microbial cell
101 walls, cellulose, plant cell walls) and additionally enhanced inorganic N supply (in
102 cellulose and plant cell wall treatments). We analyzed respiration of substrate-C as well
103 as the recovery of substrate-C in marker PLFAs specific for certain groups of
104 microorganisms in order to unravel the fate of decomposed substrates within the soil
105 microbial communities.

106

107 **Materials and Methods**

108

109 *Soil*

110 The soil for the incubation experiment originated from a 65-year-old beech forest (*Fagus*
111 *sylvatica*) about 40 km southwest of Vienna (48°07' N 16°03' E, 510 m a.s.l.). Soil was
112 classified as Dystric Cambisol over flysh (pH in CaCl₂ between 4.5 and 5.1) with a
113 mean organic carbon content of 7.45% and nitrogen content of 0.48% in the A horizon.

114 Soil was collected in February 2008 (winter community) and in June 2008 (summer
115 community and community from girdling plots). Girdling of beech trees had been
116 performed in May 2006 by removal of the bark over 10 cm sections around the
117 circumference of the stems. Experimental setup in the field, as well as microbial
118 processes and microbial communities were described in detail by Kaiser *et al.* (2010),
119 Rasche *et al.* (2010), Kaiser *et al.* (2011). Soil was stored at 4°C (winter) and 12°C
120 (summer) for two weeks until the start of the incubation experiment. Half of the winter
121 soil was transferred to 12°C for equilibration 3 days before the incubation. Soil
122 characteristics (C and N pools, functional microbial communities) of soils collected in
123 winter and in summer from control and from girdled plots were described in Koranda *et al.*
124 *et al.* (2013), microbial community composition (determined from phospholipid fatty acids)
125 is given in Table 1. Although relative abundances of major microbial groups did not differ
126 significantly between the soils, a PCA calculated from all PLFA abundances revealed
127 distinct microbial community composition of soils collected in winter, in summer from
128 control and from girdled plots (data not shown).

129

130 *Substrates*

131 Five ¹³C-labelled substrates differing in complexity and C and N content were used for
132 the incubation experiment: Glucose, protein, microbial cell walls, cellulose and plant cell
133 walls, containing 20 atom % ¹³C, except for cellulose (16 atom %) and protein (98 atom
134 %). Glucose (99 atom % ¹³C, from Sigma) and cellulose (97 atom % ¹³C, from IsoLifeBV)
135 were diluted with the respective unlabelled substances, algal protein extract (98 atom %
136 ¹³C, N content ~60%, from Sigma) was applied undiluted.

137 ¹³C-labelled microbial cell walls were prepared as follows: Two bacterial species
138 (*Pectobacterium carotovorum* and *Verrucomicrobium spinosum*) and one fungal species
139 (*Aspergillus nidulans*) were grown on ¹³C-glucose (20 atom % ¹³C). Growth conditions
140 were described by Keiblinger *et al.* (2010). Microbial biomass was dried and then
141 resuspended in NaCl-solution. After mechanical destruction of cell walls by ultrasonic
142 treatment and bead beating, residues were repeatedly extracted with NaCl-solution,
143 water, methanol/chloroform (5:3), hexane and pure water to remove all labile cell
144 constituents. The remaining residues were dried, homogenized (ball mill) and stored
145 frozen.

146 ¹³C-labelled plant cell walls were prepared as follows: ¹³C-labelled wheat roots
147 (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 $\text{NH}_4\text{NO}_3 \text{g}^{-1}$ 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 $^{13}\text{CO}_2$ 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*

181 Microbial respiration rates were measured at 5 time points during the incubation period.
182 Prior to each gas sampling, incubation tubes were sealed at the bottom, cotton wool was
183 removed and instead polypropylene tubes closed by airtight rubber caps were mounted
184 on the incubation tubes (Inselbacher et al., 2009). 15 ml of headspace gas were
185 sampled by syringe immediately after closing the tubes and replaced by 15 ml of air
186 (ambient CO₂ concentration). A second gas sample was taken after 30 minutes.
187 Concentration and carbon isotope ratio of CO₂ (relative to VPD) was determined via a
188 GasBench II interfaced to continuous-flow isotope ratio mass spectrometry (IRMS; Delta
189 V Advantage, Thermo Fisher, Germany). Respiration from substrate was calculated
190 according to the following equation:

$$191 R_{\text{substrate}} = \text{APE}^{13}\text{C}_{\text{resp}} / \text{APE}^{13}\text{C}_{\text{substrate}} * R_{\text{total}}$$

192 Where APE¹³C means atom % excess ¹³C in respiration and substrates, respectively,
193 and R_{total} is total respiration.

194

195 *Phospholipid fatty acids*

196 Phospholipid fatty acids (PLFAs) in soils were extracted by a mixture of methanol,
197 chloroform and citrate buffer (2:1:0.8, v/v/v), then separated from neutral lipids on silica
198 columns and finally subjected to alkaline methanolysis (see Koranda *et al.* (2011) for
199 details). Blanks of substrates without soils were treated similarly in order to verify that
200 substrates did not contain PLFAs. Dried fatty acid methyl esters were re-dissolved in
201 isooctane and concentrations and carbon isotope ratios of PLFAs were determined by a
202 Trace Ultra GC (Thermo Fisher) interfaced with an IRMS (Delta V Advantage, Thermo
203 Fisher) via a combustion unit (GC combustion II/TC, Thermo Fisher). A mixture of
204 FAMES (Supelco, nr. 47080-U and 47885-U) was used as a qualitative standard. An
205 internal standard (19:0) was used for calculation of FAME concentrations, as well as for
206 correction of δ¹³C values. δ¹³C values of PLFAs were also corrected for δ¹³C values of
207 the C added during methanolysis. We used the sum of the fatty acids i15:0, a15:0, i16:0,
208 i17:0, a17:0 as indicator of Gram-positive bacteria, the sum of 16:1ω9, 16:1ω7, 16:1ω5,
209 18:1ω7, 18:1ω5, cy17:0, cy19:0, cy18:0 as indicator of Gram-negative bacteria and the
210 fatty acids 15:0 and 17:0 as indicator for bacteria in general (Zelles, 1997; Leckie, 2005).
211 The PLFA 16:1ω5 has also been reported as a marker for arbuscular mycorrhizal fungi
212 (Olsson, 1999). However, as in beech forest soils ectomycorrhiza is the dominant form
213 of mycorrhiza and moreover, mycorrhizal fungi had probably decayed in our system due

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

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

$$220 C_{\text{Inc}} = \text{APE}^{13}\text{C}_{\text{PLFAs}} / \text{APE}^{13}\text{C}_{\text{substrate}} * C_{\text{PLFAs}}$$

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

223

224 *Statistics*

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

234

235

236 **Results**

237

238 *Microbial substrate utilization*

239 In all incubations a peak in substrate respiration shortly after the beginning of the
240 incubation period followed by a decline towards the end of the first day could be
241 observed, most likely because also complex substrates contained a small amount of
242 monomers (Fig. 1). Respiration of complex substrates (microbial cell walls, cellulose and
243 plant cell walls) increased from the third day until the end of the incubation period
244 (except for cellulose incubations without inorganic N).

245 Microbial communities in soils collected in winter or summer significantly differed in the
246 cumulative respiration of labile substrates (Fig. 1, Table 2). While the summer
247 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 $\mu\text{mol g}^{-1}$ 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

282 cell walls and cellulose incubations with added N. Addition of inorganic N to complex C
283 substrates strongly altered the fate of substrate C within the microbial community
284 compared to incubations without N. Factor loadings on PCA-axis 1 were highest for
285 fungal marker PLFAs and two marker PLFAs for Gram-negative bacteria (Fig. 2b).
286 Bacterial markers had highest loadings on PCA-axis 2.

287 The pattern of the distribution of substrate C within the microbial communities is further
288 illustrated by the substrate incorporation into marker PLFAs for different microbial groups
289 (in percent of total incorporated substrate C; Fig. 3). At low N availability the highest
290 proportion of total substrate derived C was generally detected in marker PLFAs for
291 Gram-negative bacteria, with especially high values being found in incubations of
292 microbial cell walls (~47%), followed by cellulose (33 - 41%) and glucose incubations
293 (~36%). Markers for Gram-positive bacteria incorporated the highest proportion of
294 substrate C in cellulose incubations (between 20 and 27%). Highest substrate
295 incorporation in fungal markers was detected in incubations of glucose (20-23 % of total
296 incorporated C) and plant cell walls (17-20 %). In winter fungal markers incorporated a
297 significantly higher proportion of protein than in summer (19% compared to 15.3%,
298 respectively).

299 Inorganic N addition not only increased microbial utilization of complex C substrates
300 (Table 2), but also altered the proportional substrate utilization of microbial groups (Fig.
301 3). While the proportion of substrate C in fungal markers was significantly enhanced by
302 N addition to cellulose and plant cell wall incubations, the proportion of substrate
303 incorporation in Gram-negative bacteria declined (as well as substrate incorporation in
304 Gram-positive bacteria in cellulose incubations of soil from girdled plots). The increase
305 in fungal substrate incorporation by enhanced N availability was significantly stronger in
306 soil from girdled plots than from control plots ($p < 0.001$ and $p < 0.05$, respectively),
307 substrate C in fungal markers accounting for 70 % and 52 % of total recovered substrate
308 C in N-amended cellulose and plant cell wall incubations of soil from girdled plots,
309 respectively. In cellulose incubations of control soil the observed decline in relative
310 bacterial substrate incorporation by N addition was mainly due to a 20-fold increase in
311 absolute fungal substrate incorporation, while absolute bacterial substrate utilization was
312 not significantly changed (Table 3). In cellulose incubations of soil from girdled plots,
313 however, a dramatic increase in fungal activity by N addition (85-fold increase in ^{13}C
314 incorporation) was accompanied by a significant decline in absolute substrate
315 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 *¹³C incorporation into specific phospholipid fatty acids*

324 ¹³C from glucose was detected in nearly all the analyzed PLFAs (Fig. 4a), highest $\delta^{13}\text{C}$ -
325 values being measured in the fungal marker 18:2 ω 6 in soil from girdled plots. It has to
326 be kept in mind that $\delta^{13}\text{C}$ -values depict the ¹³C incorporation into PLFAs relative to total
327 C in PLFAs (thus normalizing differences in abundances of PLFAs) and not the total ¹³C-
328 incorporation into PLFAs (given in Fig. S1).

329 In protein incubations (Fig. 4b), substrate ¹³C was also recovered in most of the fatty
330 acids, similar to the pattern in glucose incubations. Highest $\delta^{13}\text{C}$ -values were measured
331 in soil collected in winter in the protozoan or fungal marker 20:4 ω 6, 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 i17:1 ω 8.

334 The pattern of $\delta^{13}\text{C}$ -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: 20:4 ω 6, 16:0, and two markers for
337 Gram-negative bacteria, 16:1 ω 7 and 18:1 ω 7.

338 $\delta^{13}\text{C}$ -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 16:1 ω 5 (Gram-negative bacteria) in soil from
341 girdled plots, while in plant cell wall incubations highest $\delta^{13}\text{C}$ -values were measured in
342 the fungal markers 18:2 ω 6 and 18:3 ω 3. ¹³C-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 ¹³C-incorporation from cellulose in PLFA 16:1 ω 5 nearly
346 dropped to zero at high N availability. According to changes in $\delta^{13}\text{C}$ -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 $\delta^{13}\text{C}$ -values of the PLFAs i15:0 and i17:0 were decreased by N

350 addition in both cellulose and plant cell wall incubations, $\delta^{13}\text{C}$ -values of i16:0 and a17:0
351 were increased.

352

353

354 **Discussion**

355

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

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

381 Degradation of complex C substrates was higher in soils from girdled plots than from
382 control plots (Table 2), possibly indicating microbial adaptation to high amounts of dead
383 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 16:1 ω 5, 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

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470

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472

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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 ($\mu\text{g g}^{-1}$ DW)			Substrate derived C in PLFAs ($\mu\text{g g}^{-1}$ 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 ($\mu\text{g g}^{-1}$ DW)						
Winter	<i>Gram- bacteria</i>		<i>Gram+ bacteria</i>		<i>Fungi</i>	
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	<i>Gram- bacteria</i>		<i>Gram+ bacteria</i>		<i>Fungi</i>	
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	<i>Gram- bacteria</i>		<i>Gram+ bacteria</i>		<i>Fungi</i>	
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 \pm 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 \pm 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 \pm SE (n = 3).

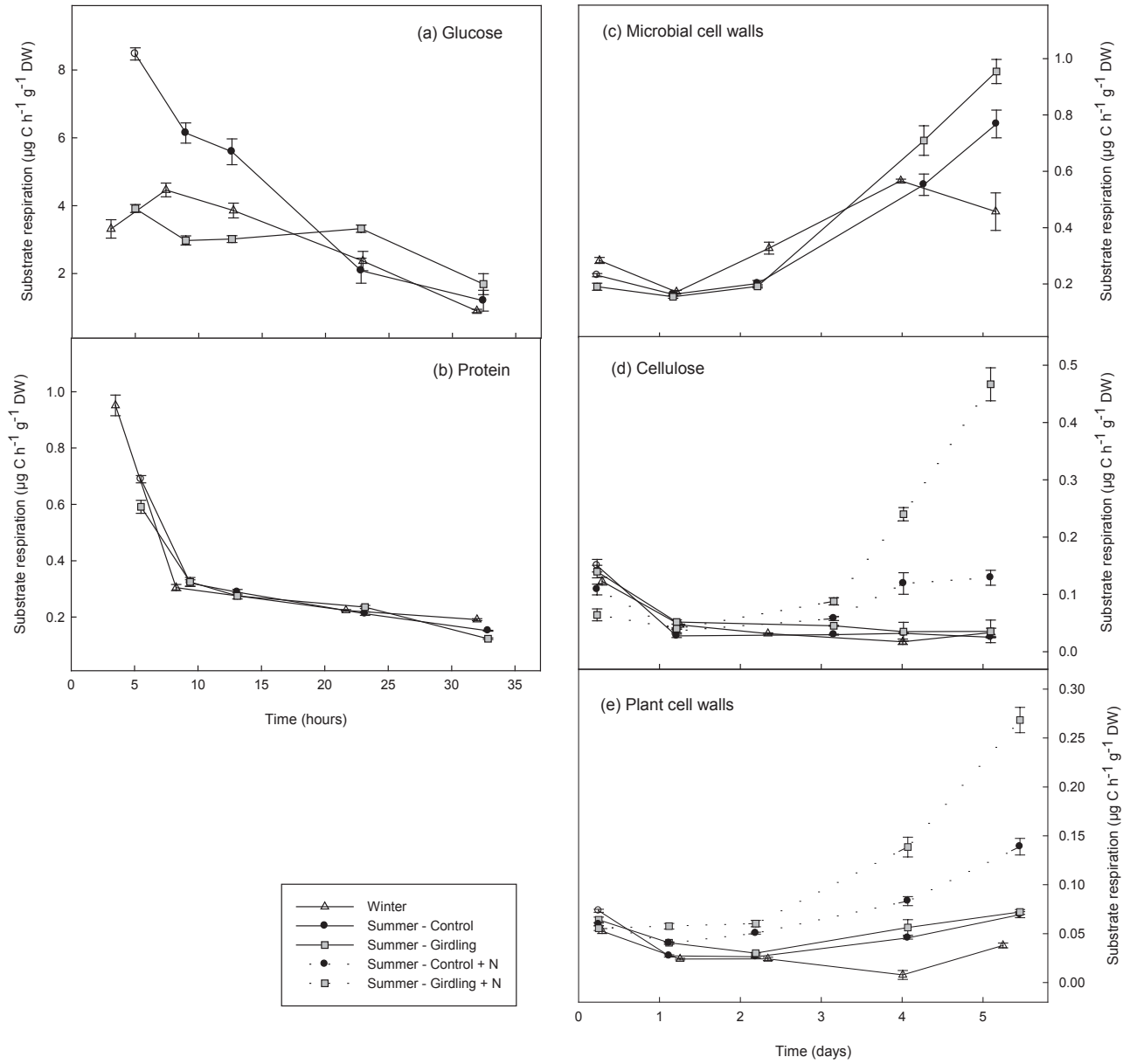


Fig. 1

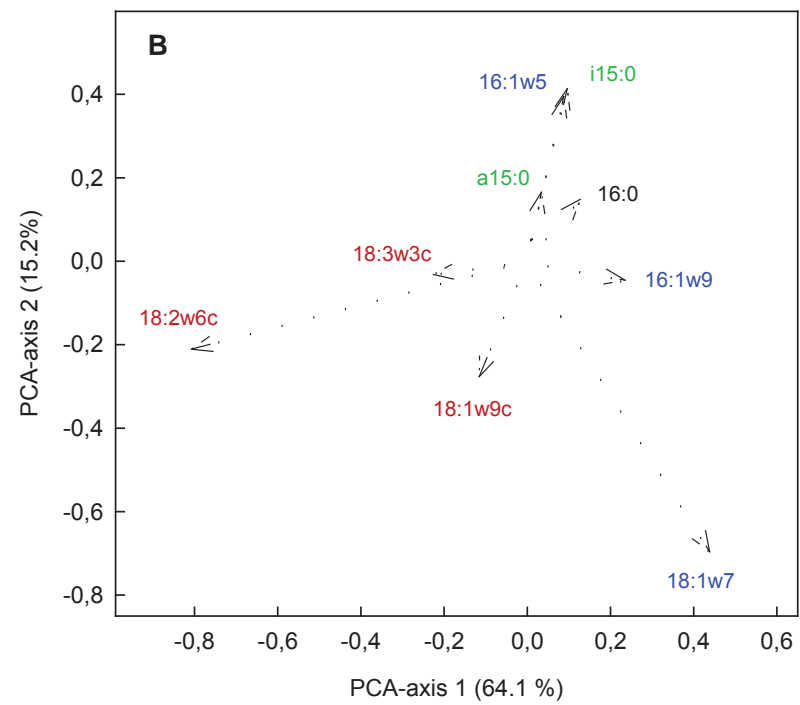
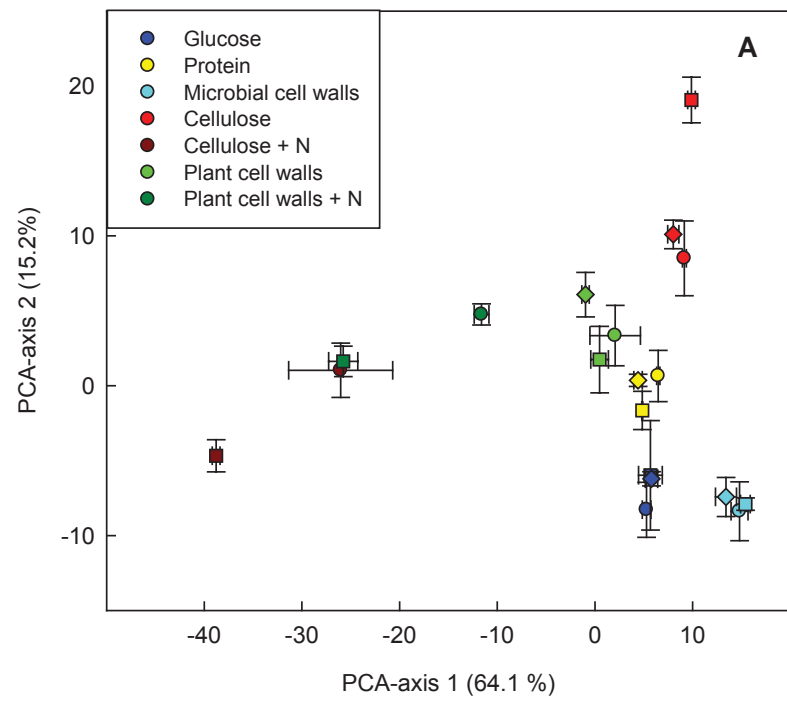


Fig. 2

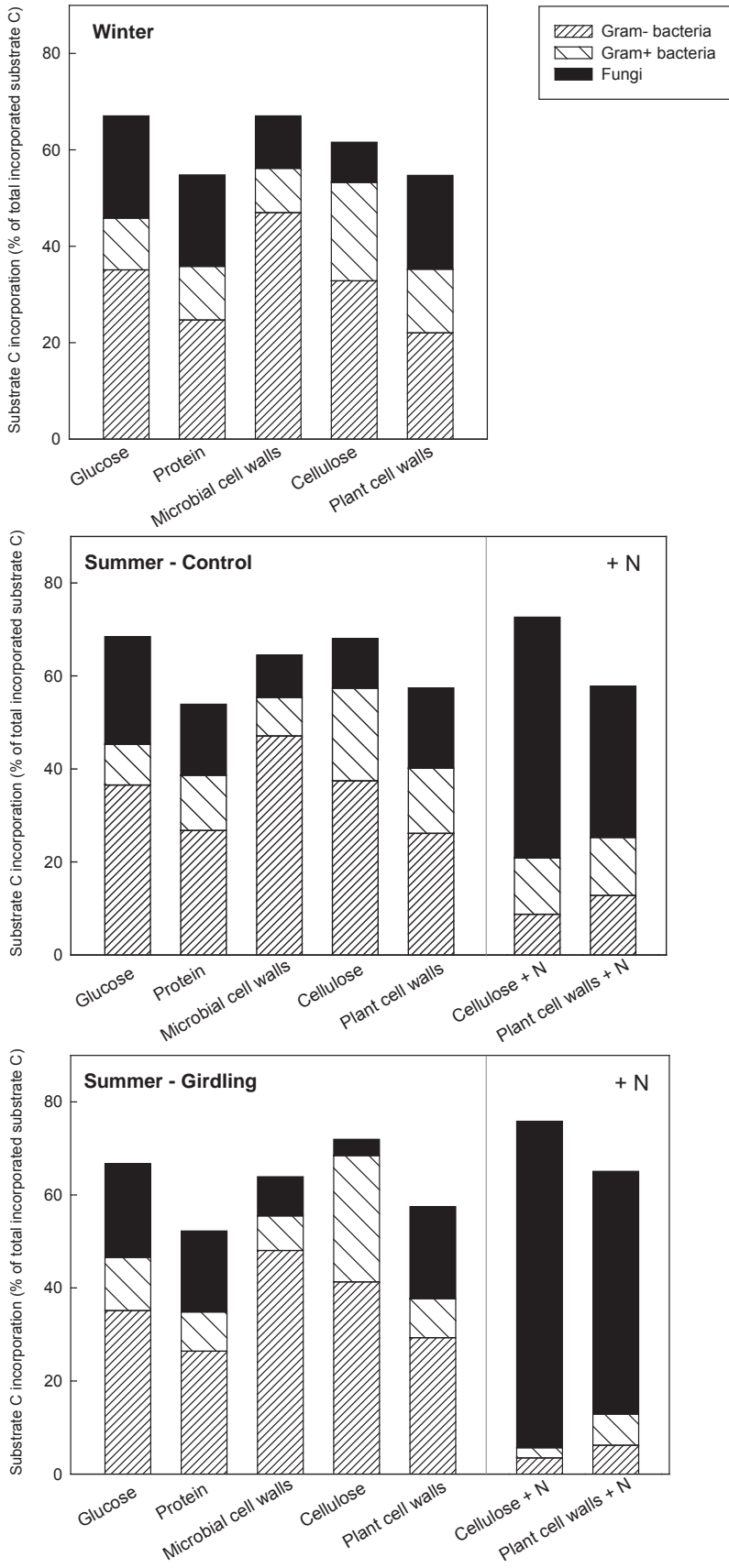


Fig. 3

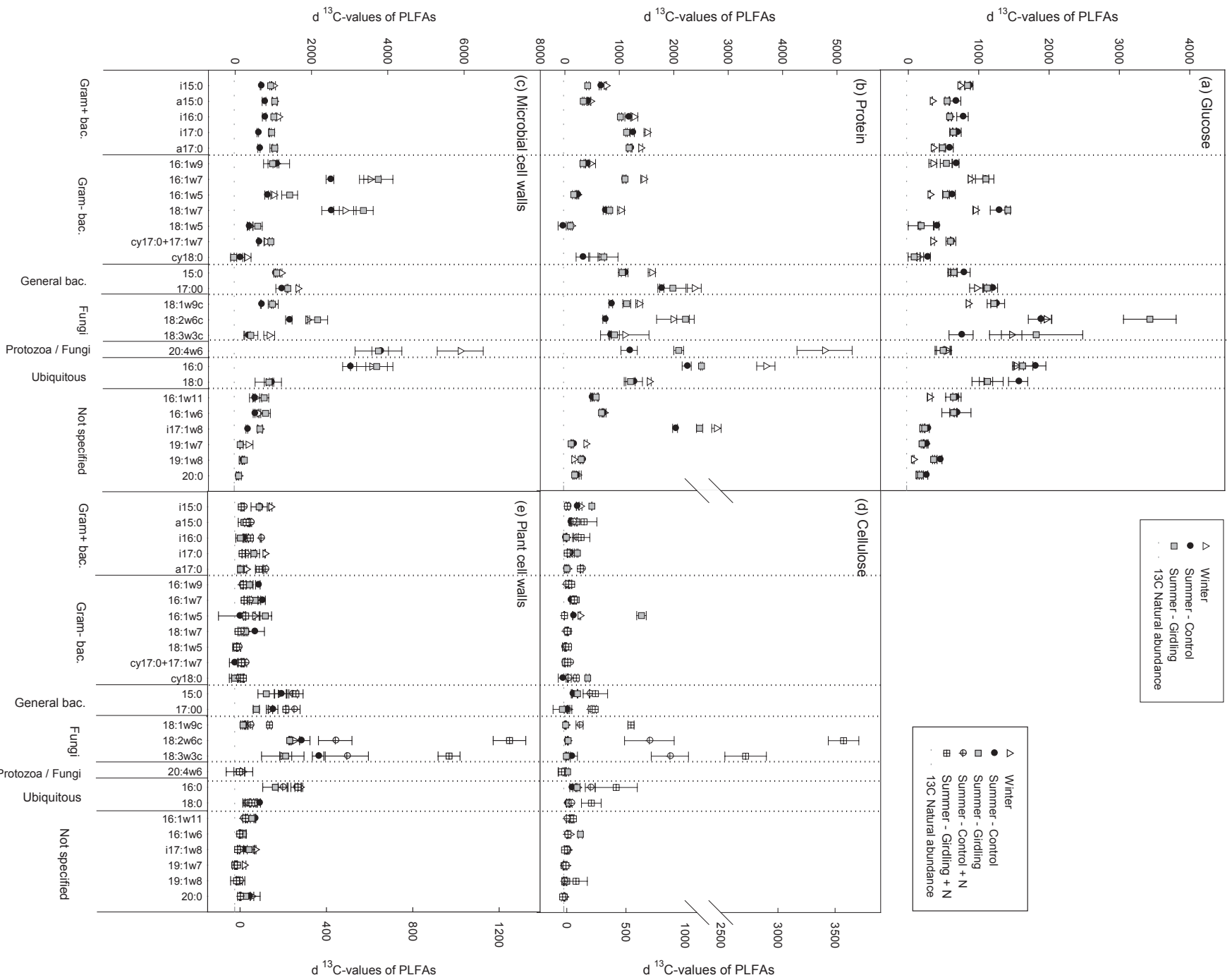


Fig. 4

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.

	Total bacteria		Gram+ bacteria		Gram- bacteria		Fungi	
Winter								
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)

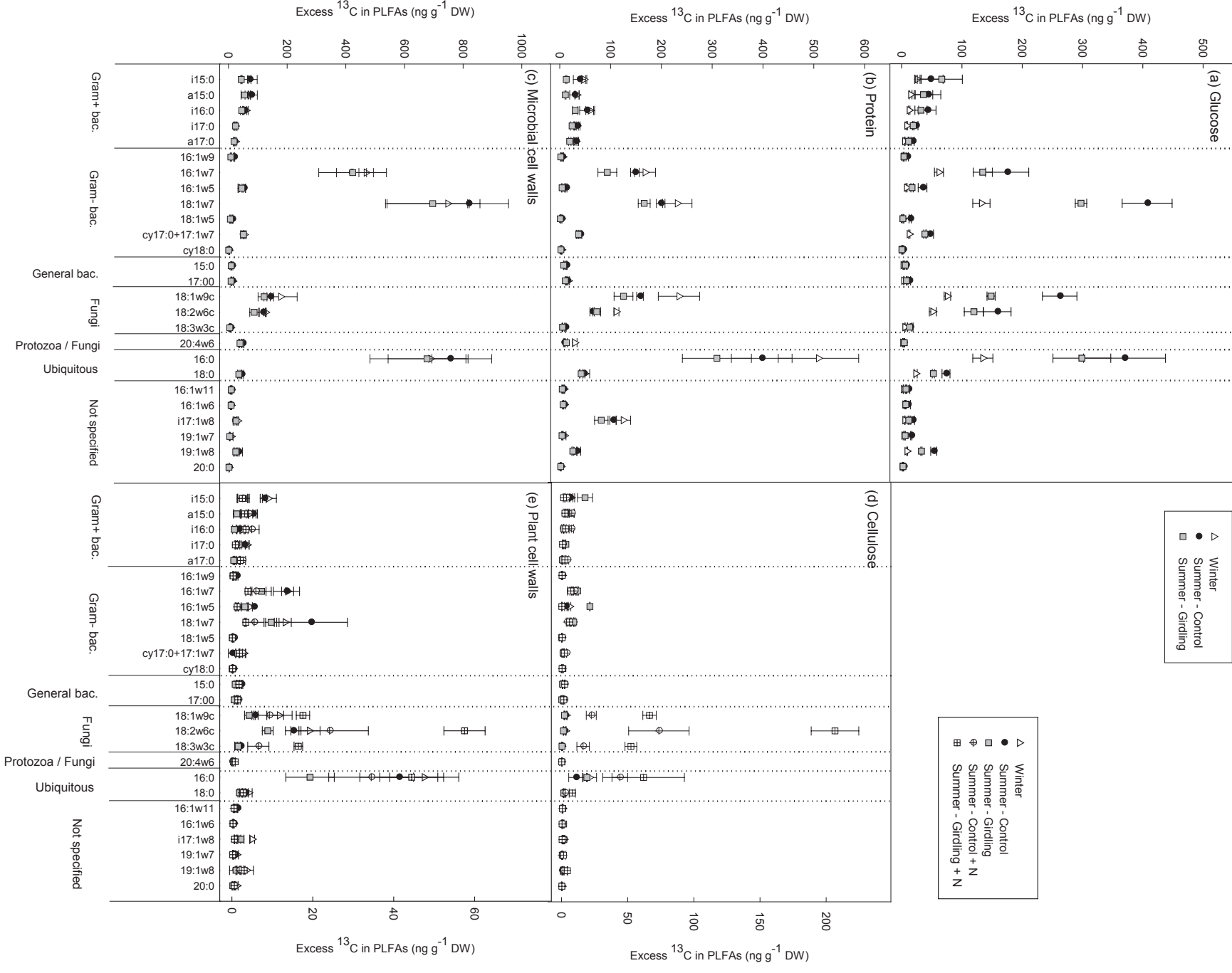


Figure S1. ^{13}C incorporation into PLFAs in soils collected in winter (triangles), in summer from control plots (circles) and from girdled plots (squares), incubated with five organic substrates with or without inorganic N addition. Values are means \pm SE (n = 3).