Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

Biogeosciences Discussions
Open Access
EGU

Carbon turnover in cell compartments and microbial groups in soil

Anna Gunina[1,2], Michaela Dippold[1], Bruno Glaser[3], Yakov Kuzyakov[1,4]

[1] Department of Agricultural Soil Science, Georg-August-University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany

[2] Department of Soil Biology and Biochemistry, Dokuchaev Soil Science Institute, Russian Federation;

[3] Department of Soil Biogeochemistry, Institute of Agricultural and Nutritional Science, Martin-Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, 06120 Halle (Saale), Germany

[4] Department of Soil Science of Temperate Ecosystems, Georg-August-University of Göttingen, Büsgenweg 2, 37077, Göttingen, Germany

Corresponding Author:
Anna Gunina
Department of Agricultural Soil Science
Georg-August-University of Göttingen
Büsgenweg 2
37077 Göttingen
Tel: 0551/39-20502
email: guninaann@gmail.com
Tel.: 0157/85566093

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

Abstract

Microorganisms regulate the carbon (C) cycle in soil, controlling the utilization and recycling of organic substances. To reveal the contribution of particular microbial groups to C utilization and C turnover within the microbial cells, fate of ^{13}C-labeled glucose was studied under field conditions. The ^{13}C was traced in cytosolic substances, amino sugars and phospholipid fatty acids (PLFA) at intervals of 3, 10 and 50 days after glucose addition.

^{13}C enrichment into PLFA (~1.5% of PLFA C at day 3) was one order of magnitude greater than into the cytosol, showing the importance of cell membranes for initial C utilization. ^{13}C enrichment of amino sugars in living microorganisms at day 3 accounted for 0.57%, resulting that the turnover of cell wall components is two times slower than that of cell membranes. Turnover time of C in the cytosol (150 days) was three times longer than in PLFAs (47 days). Consequently, despite the lability of cytosol pool and expected fast turnover rates, intensive recycling of cytosol components, within the living cells, leads to a longer turnover time. Amino sugars originate mainly from microbial residues, thus longer experimental periods are required for estimation of their turnover times.

Both PLFA and amino sugar profiles indicated that glucose C was preferentially used by bacteria. The ^{13}C incorporated into bacterial cell membrane components decreased with time, but it remained constant or even increased for filamentous microorganisms. Hence, over a short period, bacteria contribute more to the utilization of low molecular weight organic substances, whereas filamentous microorganisms are responsible for further C transformations. Thus, tracing ^{13}C in cellular compounds with contrasting turnover rates elucidated the role of microbial groups and their cellular compartments in C utilization and recycling in soil. This information is especially important for assessing C fluxes in soil and the contribution of C from microbial residues to soil organic matter.

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

Keywords

Microbial biomarkers; phospholipid fatty acids; amino sugars; ^{13}C labeling; glucose utilisation; soil microbial biomass.

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

1. Introduction

Over the last decade, numerous studies have demonstrated the role of soil microorganisms in regulating the fate and transformation of organic compounds. Soil microorganisms produce exoenzymes to carry out the primary degradation of plant as well as microbial polymers to monomers. Further transformations of monomers then take place within the microbial cells. Monomeric substances pass into the living microbial pool and are partly mineralised to CO_2, while part is assimilated into cell polymers and ultimately incorporated into soil organic matter (SOM) after cell death (Kindler et al., 2006). Understanding the fate of substances originated from plants and microbial residues into living biomass is therefore crucial for estimating the recycling of carbon (C) in soil and its stabilization as SOM.

Living microbial biomass (MB) is a highly active and heterogeneous pool, although it accounts for only 2-4% of the total SOM (Jenkinson and Ladd, 1981). Heterogeneity is evident at the level of single cells in the various cellular compartments with different properties, structures and biochemistry: from the highly heterogeneous cytosol (Malik et al., 2013), to well-structured cell membranes and cell walls. Due to their chemical composition and spatial localization, compounds of cell membranes (phospholipid fatty acids (PLFAs)) and cell walls (amino sugars) have different turnover times within the cell as well as different stabilities within SOM.

Organic compounds that are taken up by microorganisms first enter the cytosol (Bremer and Kuikman, 1994), which is presumed to be the most dynamic pool within microbial cells. However, due to the heterogeneity of this pool, no single C turnover time can be estimated. The calculated turnover time of intact PLFAs in soil after microbial death is 2.8 days (Kindler et al., 2009), resulting PLFAs are mainly used to characterize the living microorganisms (Frostegard et al., 2011; Rethemeyer, 2004). However, no data concerning turnover time of PLFA C in the living biomass are currently published. The formation of

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

amino sugars from plant biomass is relatively rapid at 6.2–9.0 days (Bai et al., 2013), whereas their turnover times in soil vary between 6.5–81.0 y^{-1} (Glaser et al., 2006). Thus, PLFAs and amino sugars can be used to trace the fate of C within the living microorganisms and estimate their contribution to SOM (Schmidt et al., 2007).

Some cell compartments, such as the cytoplasm, are not specific for various microbial groups, whereas phospholipids are partly specific and consequently can be used to estimate microbial community structure. Thus, PLFAs of bacterial (i16:0, a16:0, i15:0, a15:0, 16:1ω7, 18:1ω7) and fungal communities (18:2ω6,9; 18:3ω6,9,12; 16:1ω5) are used to draw conclusions about the qualitative composition of living microbial communities, their contribution to utilisation of C by various origin (plant or microbial) and to understand trophic interactions within the soil (Ruess et al., 2005). In contrast, amino sugars (glucosamine, galactosamine, mannosamine and muramic acid) are usually used to assess the contributions of bacterial and fungal residues to SOM (Engelking et al., 2007; Glaser et al., 2004). Muramic acid is of bacterial origin, whereas glucosamine is derived from both fungal and bacterial cell walls (Glaser et al., 2004). Galactosamine is more abundant in fungal than in bacterial cell walls (Engelking et al., 2007; Glaser et al., 2004).

Fate of cell membrane and cell wall biomarkers in soil is strongly linked to the turnover of microorganisms. The cellular turnover of the soil bacterial community is higher (ca. 2–3-fold per year) than that of the fungal community (ca. 0.75 times per year) (Moore et al., 2005; Rousk and Baath, 2007; Waring et al., 2013). However, the relationship between cellular turnover and intracellular C turnover – the question of ecological relevance for the C cycle – has rarely been investigated. Therefore, if PLFAs characterize the living microbial community and are rapidly decomposed after cell death (Kindler et al., 2009), a similar degradation can be assumed for PLFAs molecules originating from various microorganisms. In contrast, amino sugar polymers display markedly different decomposition kinetics, in that

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

105 they can be stabilized in SOM as polymers (Glaser et al., 2004). Thus, the comparison of C
106 turnover for cell membrane and cell wall components can be used to characterize the
107 contribution of various microbial groups to short-term C utilisation and to the stabilization of
108 microbially derived C in SOM.

109 Combination of PLFAs and amino-sugar biomarkers analyses, as well as cytosolic C
110 measurement with isotope tracing techniques (based on ^{13}C natural abundance or $^{13}C/^{14}C$
111 labelling) have been used in various studies to characterize organic C utilisation by the
112 microbial community (Bai et al., 2013; Brant et al., 2006). However, to date no systematic
113 studies have compared these contrasting cell compartments in a single soil within a C
114 turnover experiment. Therefore, this study aimed to examine C allocation to various cell
115 compartments following ^{13}C labelling with a ubiquitous monomer, glucose. Glucose has a
116 higher concentrations in the soil solution compared to other low molecular weight organics
117 (Fischer et al., 2007), due to its diverse origin: from cellulose decomposition, presence in
118 rhizodeposition (Derrien et al., 2004; Gunina and Kuzyakov, 2015), and synthesis by
119 microorganisms. It is also used by most of the microbial groups, and, thus, is the most
120 suitable substance for such a study.

121 We analyzed glucose derived ^{13}C partitioning into the cytosol, cell membranes and
122 cell walls, to evaluate the turnover time of C in each pool, and to assess the contribution of
123 bacterial and fungal biomass to SOM. We hypothesized that: 1) C from sugars is first
124 incorporated into the cytosol, and subsequently into structural compartments such as cell
125 membranes (PLFA) and cell walls (amino sugars). Thus, the turnover times should increase
126 in the order: cytosol < PLFA < amino sugars; 2) incorporation of ^{13}C glucose should be faster
127 and higher for bacterial than for fungal biomarkers, because bacterial biomass has a faster
128 cell turnover than fungal biomass; 3) due to amino sugars have long turnover times and are

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

129 mainly dominated in microbial necromass, all incorporated ^{13}C can be related only to living
130 biomass and allow estimate percent of replaced C in amino sugars of living microorganisms.

131

132 **2. Material and Methods**

133 *2.1. Field site and experimental design*

134 The ^{13}C labeling field experiment was established at an agricultural field trial in Hohenpölz,
135 Germany (49°54'N, 11°08'E, at 500 m a.s.l.). Triticale, wheat and barley were cultivated by a
136 rotation at the chosen site. The soil type was a loamy haplic Luvisol (IUSS Working group
137 WRB, 2014) and had the following chemical properties in the uppermost 10 cm: total organic
138 C content 1.5%, C/N 10.7, pH 6.6, clay content 22%, CEC 13 $cmol_C$ kg^{-1}. The annual
139 precipitation is 870 mm and mean annual temperature is +7 °C.

140 In summer 2010, following harvest of the triticale, columns (diameter 10 cm and
141 height 13 cm) were installed to a depth of 10 cm. Each column contained 1.5 kg of soil. The
142 50 mL of uniformly labelled ^{13}C glucose (99 atom % ^{13}C) was injected into the columns via a
143 syringe at five points inside the column to spread the tracer homogeneously. Syringe was
144 equipped with a special pipe having length 13 cm and perforated along the whole length,
145 while the end of the pipe was sealed to prevent glucose injection below of the column. Each
146 column received 93.4 µmol ^{13}C of tracer (0.06 µmol ^{13}C g^{-1}soil) and similar amounts of non-
147 labeled glucose were applied to the control columns. The experiment was done in four field
148 replicates, which were organized in a randomized block design. Labelled and control columns
149 were present within each block. For the first 10 days of the experiment the rainfall was
150 excluded by protective shelter, which was then removed and the experiment was run for 50
151 days in total. After 3, 10 and 50 days, separate soil columns (four columns where ^{13}C was
152 applied and four control columns) were destructively sampled.

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

153 The soil was removed from the column, weighed and the water content was
154 determined in a subsample. Soil moisture was determined by drying samples for 24 h at 105
155 °C and was essentially constant during the experiment, ranging between 21–25%. Each soil
156 sample was sieved to <2 mm and divided into three parts. One part was stored frozen (-20ºC)
157 for PLFA analysis, another was cooled (+5°C) (during one week) before the microbial
158 biomass analysis, and the rest was freeze-dried and used for amino-sugar analysis and for
159 measurement of the total amount of glucose derived ^{13}C remaining in the soil.

160

161 *2.2. Bulk soil $\delta^{13}C$ analysis*

162 The soil for the $\delta^{13}C$ analysis was milled and $\delta^{13}C$ values of bulk SOM were determined
163 using a Euro EA Elemental Analyser (Eurovector, Milan, Italy) unit coupled via a ConFlo III
164 interface (Thermo-Fischer, Bremen, Germany) to a Delta V Advantage IRMS (Thermo
165 Fischer, Bremen, Germany). The amount of glucose derived ^{13}C remaining in the soil was
166 calculated based on a mixing model (Equations 1 and 2), where the amount of C in the
167 background sample in Equation 1 was substituted according to Equation 2.

168 $$[C]_{soil} \cdot at\%_{soil} = [C]_{BG} \cdot at\%_{BG} + [C]_{glc} \cdot at\%_{glc} \quad \text{Eq. (1)}$$

169 $$[C]_{soil} = [C]_{BG} + [C]_{glc} \quad \text{Eq. (2)}$$

170 with:

171 $[C]_{soil/BG/glc}$ C amount of enriched soil sample / background soil sample /
172 glucose derived C in soil ($mol \cdot g_{soil}^{-1}$)
173 $at\%_{soil/BG/glc}$ ^{13}C in enriched soil sample / background soil sample /
174 applied glucose (at%)

175

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

176 *2.3. Cytosolic C pool*

177 The cytosolic pool was determined by the fumigation–extraction technique from fresh soil
178 shortly after sampling, according to Wu et al. (1990) with slight changes. Briefly, 15 g fresh
179 soil was placed into glass vials, which were exposed to chloroform during 5 days. After
180 defumigation, the cytosolic C was extracted from the soil with 45 mL 0.05 M K_2SO_4. Due to
181 fumigation–extraction technique allows to obtain not only soluble components, but also cell
182 organelles and cell particles, we named pool of C in fumigated extracts as cytosol only for
183 simplification of terminology. Organic C was measured with a high-temperature combustion
184 TOC-analyser (Analyser multi N/C 2100, Analytik Jena, Germany). The cytosolic pool was
185 calculated as the difference between organic C in fumigated and unfumigated samples
186 without correcting for extraction factor. After organic C concentration were measured, the
187 K_2SO_4 extracts were freeze-dried and the $\delta^{13}C$ values of a 30–35 µg subsample were
188 determined using EA-IRMS (instrumentation identical to soil $\delta^{13}C$ determination). The
189 amount of glucose derived ^{13}C in fumigated and unfumigated samples was calculated
190 according to the above-mentioned mixing model (Equations 1 and 2). The ^{13}C in the
191 microbial cytosol was calculated from the difference in these incorporations.

192

193 *2.4. Phospholipid fatty acid analysis*

194 The PLFA analysis was performed using the liquid–liquid extraction method of Frostegard et
195 al. (1991) with some modifications (Gunina et al., 2014). Briefly, 6 g soil were extracted with
196 a 25-mL one-phase mixture of chloroform, methanol and 0.15 M aqueous citric acid (1:2:0.8
197 v/v/v) with two extraction steps. The 19:0-phospholipid (dinonadecanoylglycerol-
198 phosphatidylcholine, Larodan Lipids, Malmö, Sweden) was used as internal standard one
199 (IS1) and was added directly to soil before extraction (25 µL with 1 µg μL^{-1}). Additional

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

chloroform and citric acid was added to the extract achieve a separation of two liquid phases, in which the lipid fraction was separated from other organics. Phospholipids were separated from neutral- and glycolipids by soild-phase extraction using a silica column. Alkaline saponification of the purified phospholipids was performed with 0.5 mL 0.5 M NaOH dissolved in dried MeOH, followed by methylation with 0.75 mL BF_3 in methanol. The resulting fatty acid methyl esters (FAMEs) were purified by liquid–liquid extraction with hexane (three times). Before the final quality and quantity measurements, internal standard two (IS2) (13:0 FAME) (15 µL with 1 µg μL^{-1}) was added to the samples (Knapp, 1979).

All PLFA samples were analysed by gas chromatograph (GC) (Hewlett Packard 5890 GC coupled to a mass-selective detector 5971A) (Gunina et al., 2014). A 25 m HP-1 methylpolysiloxane column (internal diameter 0.25 mm, film thickness 0.25 µm) was used (Gunina et al., 2014). Peaks were integrated and the ratio to IS2 was calculated for each peak per chromatogram. Substances were quantified using a calibration curve, which was constructed using 29 single standard substances, (13:0, 14:0, i14:0, a14:0, 14:1ω5, 15:0, i15:0, a15:0; 16:0, a16:0, i16:0, 16:1ω5; 16:1ω7, 10Me16:0, 17:0, a17:0, i17:0, cy17:0, 18:0, 10Me18:0, 18:1ω7, 18:1ω9, 18:2ω6,9, 18:3ω6,9,12, cy19:0, 19:0, 20:0, 20:1ω9, 20:4ω6) at six concentrations. The recovery of extracted PLFA was calculated using IS1 and the PLFA contents of samples were individually corrected for recovery. Based on the measured PLFAs contents, the PLFAs C was calculated for the each single compound.

The $^{13}C/^{12}C$ isotope ratios of the single fatty acids were determined by an IRMS Delta PlusTM coupled to a gas chromatograph (GC; Trace GC 2000) via a GC-II/III-combustion interface (all units from Thermo-Fisher, Bremen, Germany) (Gunina et al., 2014). A 15 m HP-1 methylpolysiloxane column coupled with a 30 m HP-5 (5% Phenyl)-methylpolysiloxane column (both with an internal diameter of 0.25 mm and a film thickness of 0.25 µm) were used. The measured $\delta^{13}C$ values of the fatty acids were corrected for the

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

effect of derivative C by analogy to Glaser and Amelung (2002) and were referenced to Pee Dee Belemnite by external standards. The enrichment of ^{13}C in single fatty acids was calculated by analogy to bulk soil and cytosol according to Equations 1 and 2, following a two-pool dilution model (Gearing et al., 1991).

2.5. Amino sugar analysis

Acid hydrolysis was performed to obtain amino sugars from soil and further ion removal was performed according to the method of Zhang and Amelung (1996) with optimization for $\delta^{13}C$ determination (Glaser and Gross, 2005). Methylglucamine (100 µL, 5 mg mL^{-1}) was used as IS1 and was added to the samples after hydrolysis. Following iron and salt removal, non-cationic compounds such as monosaccharides and carboxylic acids were removed from the extracts using a cation exchange column (AG 50W-X8 Resin, H^+ form, mesh size 100–200, Biorad, Munich, Germany) (Indorf et al., 2012). For final measurement, IS2 – fructose (50 µL, 1 mg mL^{-1}) – was added to each sample. The amino sugar content and ^{13}C enrichment were determined by LC-O-IRMS (ICS-5000 SP ion chromatography system coupled by an LC IsoLink to a Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo-Fischer, Bremen, Germany)) (Dippold et al., 2014). Amino sugars were quantified using a calibration curve, which was constructed using four single standard substances (glucosamine, galactosamine, mannosamine and muramic acid) as external standards at four different concentrations (Dippold et al., 2014).

2.6. Calculations and statistical analysis

The assignment of fatty acids to distinct microbial groups was performed by factor analysis with the principal component extraction method in combination with databases (Zelles,

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

1997). This method enables quality separation of microbial groups within the soils (Apostel et al., 2013; Gunina et al., 2014). The results of the factor analysis are presented in Supplementary Table 1.

Total incorporation of glucose derived ^{13}C ($^{13}C_{incorp}$) (means ^{13}C incorporation represented as % of total applied ^{13}C) and enrichment ($^{13}C_{enrichm}$) (means ^{13}C incorporation represented as % of total C pool) of the cytosol, PLFAs and amino sugars was calculated according to Equations 3 and 4, respectively. The C turnover time in the cell pools was calculated as $^{1}/_{k}$; the value of k was obtained from Equation 5.

$$^{13}C_{incorp} = \frac{C_{Glc}}{^{13}C_{Applied}} \times 100\% \qquad \text{Eq. (3)}$$

$$^{13}C_{enrichm} = \frac{C_{Glc}}{^{Total}C_{Pool}} \times 100\% \qquad \text{Eq. (4)}$$

with

C_{Glc}	amount of glucose derived C incorporated into a distinct cell compartment calculated by equation (1) and (2)	(µmol ^{13}C per column)
$^{13}C_{Applied}$	amount of applied glucose ^{13}C	(µmol ^{13}C per column)
$^{Total}C_{Pool}$	amount of pool C	(µmol C per column)

$$C_{enrichm(t)} = C_{enrichm(0)} \cdot \exp^{-kt} \qquad \text{Eq. (5)}$$

with

$C_{enrichm\ (t)}$	^{13}C enrichment of the compartment, obtained from Eq. 4 at time t	(%)
$C_{enrichm\ (0)}$	^{13}C enrichment of the compartment obtained from Eq. 4 at time 0	(%)

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

k	decomposition rate constant	(% day^{-1})
t	time	(days)

One-way ANOVA was used to estimate the significance of differences in total ^{13}C incorporation and enrichment of cytosol, PLFAs and amino sugars. The data always represent the mean of four replications ± standard error. To calculate the turnover time of C in the cytosol, PLFA and amino sugar pools, a single exponential model was used (Eq. 5) (Kuzyakov, 2011; Parton et al., 1987).

3. Results

3.1. Glucose utilisation and its partitioning within microbial biomass pools

Amino sugars were the largest pool, due to their accumulation in SOM, whereas pools that mainly characterize living MB showed smaller C contents (Table 1). The cytosolic pool (C content 210±7.10 for day 3; 195±14.8 for day 10; 198±19.9 mg C kg^{-1} soil for day 50) as well as nearly all PLFA groups (Suppl. Table 2) remained constant during the experiment.

[Table 1]

The cytosolic pool contained the highest amount of ^{13}C among the investigated microbial pools (15–25% of applied ^{13}C), whereas the lowest amount was recovered in amino sugars (0.8–1.6% of applied ^{13}C) (Fig. 1). The amount of glucose derived ^{13}C in the cytosolic pool decreased over time, with the largest decrease from day 3 to day 10, and then remained constant for the following month (Fig. 1). The total ^{13}C incorporation into PLFA was generally very low and was in the same range as incorporation into amino sugars (Fig. 1).

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

294 The ^{13}C dynamics in PLFA showed no clear trend (high standard error) (Fig. 1). In contrast,
295 ^{13}C in amino sugars increased two fold during the 50 day experiment ($p<0.05$).

296 **[Fig. 1]**

297

298 *3.2. Turnover time of C in microbial biomass pools*

299 To evaluate C turnover in the cytosol, PLFAs and amino sugars, we calculated the
300 enrichment (% of incorporated ^{13}C relatively to pool C) of each pool by glucose derived ^{13}C.
301 The enrichment was the highest in PLFAs (Fig. 2) and was 5–8 times lower in the cytosolic
302 pool. The ^{13}C enrichment in amino sugars was the lowest (Fig. 2). Based on the decrease of
303 ^{13}C enrichment over time (Fig. 2), the C turnover in the cytosol and PLFAs was calculated as
304 151 and 47 days, respectively. The C turnover time in the amino-sugar pool could not be
305 calculated by this approach because the maximum incorporation had not yet been reached,
306 and consequently a decomposition function could not be fitted.

307 **[Fig. 2]**

308

309 *3.3 Phospholipid fatty acids*

310 Fatty acids of bacterial origin dominated over those of fungal origin within the living
311 microbial community characterized by PLFA composition (Table 1). Gram-negative (G-)
312 fatty acids were more abundant than gram-positive (G+) ones. Actinomycetes and vesicular
313 arbuscular mycorrhiza (VAM) fatty acids dominated in the composition of filamentous
314 microorganisms, and saprotrophic fungi showed a relatively low presence in PLFAs. The
315 PLFA content of most groups did not change significantly during the experiment, reflecting
316 steady-state conditions for the microbial community (see supplementary Table 2).

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

317 Glucose derived ^{13}C was incorporated in higher portions into bacterial than into fungal
318 PLFAs (Fig. 3, top). Remarkably, the ^{13}C enrichment decreased over time for all bacterial
319 PLFAs, whereas it increased or remained constant for VAM, fungi and filamentous, bacterial
320 actinomycetes (Fig. 3, bottom), indicating differences in C turnover in single-celled
321 organisms compared to filamentous organisms.

322 **[Fig. 3]**

323

324 *3.4. Amino sugars*

325 The content of amino sugars followed the order: muramic acid < galactosamine <
326 glucosamine (Table 1). The glucosamine/muramic acid ratio varied between 17 and 55,
327 whereas the galactosamine/muramic acid ratio ranged between 12 and 19 (Table 1). This
328 provides evidence that bacterial residues were dominant in the composition of microbial
329 residues in SOM.

330 The incorporation of glucose derived ^{13}C into amino sugars increased in the order:
331 muramic acid = galactosamine < glucosamine (Fig. 4, top) reflecting partly their pool sizes.
332 The ^{13}C incorporation showed no increase from day 3 to day 50 for any amino sugars. The
333 ratios of glucosamine/muramic acid and galactosamine/muramic acid, calculated for the
334 incorporated ^{13}C, were about six. This is much lower than the ratio observed for the pools of
335 amino sugars. The ^{13}C enrichment did not increase from day 3 to day 50 for any of the amino
336 sugars. The highest enrichment was observed for muramic acid and the lowest for
337 galactosamine (Fig. 4, bottom). The ^{13}C enrichment in amino sugars was 10–20 times lower
338 than for PLFA.

339 **[Fig. 4]**

340

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

4. Discussion

4.1. Glucose decomposition

The amount of glucose derived ^{13}C remaining in soil after 50 days was in the range 80 % which was higher than reported by other studies. Glanville et al. (2012) observed that 50% of glucose C remained in SOM after 20 days; Wu et al. (1993) reported that 55% of glucose derived ^{14}C remained after 50 days; Perelo and Munch (2005) reported the mineralisation of 50% of ^{13}C glucose within 98 days. The amounts of applied C, as well as differences in microbial activity in the investigated soils, explain the variation between studies in the portion of remaining glucose C. The rather high amount of remaining glucose ^{13}C observed in this study agrees with results obtained by adding less than 150 µg glucose C g^{-1} soil (2 µmol C g^{-1} soil) compared to the application of glucose at high addition rates, i.e. more than 150 µg C g^{-1} soil (Bremer and Kuikman, 1994; Schneckenberger et al., 2008). Glucose C was stored within the cells due to the starvation conditions of microbial communities, arising from the general limitation of easily accessible C sources (Bremer and Kuikman, 1994; Schimel and Weintraub, 2003) due to long term cultivation. This leads to maintenance and starvation metabolism in microorganisms (Blagodatskaya and Kuzyakov, 2013), where the use of low molecular weight organic C for energy production, and therefore its mineralisation, are strongly reduced and conservation of C within the microorganisms prevails (Bremer and Kuikman, 1994).

The decomposition of 20% of glucose derived ^{13}C within the first three days (Fig. 1) agrees with previously reported data (Boddy et al., 2007; Gregorich et al., 1991; Perelo and Munch, 2005). Glucose is decomposed in soil in two stages (Gunina and Kuzyakov, 2015): during the first one, part of glucose C is immediately mineralized to CO_2 and part is incorporated into the microbial compartments. This first stage takes place in the first day after substrate addition and is 30 times faster than the 2nd stage (Gregorich et al., 1991), during

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

366 which C incorporated into MB is further transformed and is used for microbial anabolism
367 e.g., in stable cell polymers, or stored for later catabolism (Bremer and Kuikman, 1994). A
368 significant portion of glucose derived C was stored in the non-specific pool in SOM (Fig. 1),
369 e.g., as microbial storage compounds and other cellular building blocks, which can contribute
370 to C accumulation in microbial residues (Wagner GH, 1968; Zelles et al., 1997; Lutzow et al.,
371 2006). This part cannot be extracted by the methods applied in this study. The amino sugar
372 method detects only the peptidoglycan and chitin proportions of the cell walls, whereas other
373 constituents can not be determined by this method (Glaser et al., 2004). Chloroform
374 fumigation only partially extracts the cytosolic cell compounds, as high molecular weight
375 compounds, which interact with the soil matrix, cannot be extracted with low molarity salt
376 solution.

377

378 *4.2. Partitioning of ^{13}C between cell compounds*

379 To estimate the residual amount of C derived from applied ^{13}C-labelled low molecular weight
380 organic substances (LMWOS), the ^{13}C in SOM or in the total MB pool is frequently
381 determined. This approach, however, does not allow the portions of ^{13}C incorporated into
382 stable and non-stable C pools to be estimated, because the ^{13}C in SOM includes the sum of
383 ^{13}C in living biomass and ^{13}C in microbial residues. Furthermore, the living MB contains cell
384 compartments with a broad spectrum of C turnover times. The approach applied in the
385 present study allows the partitioning of glucose derived C in living MB to be estimated, as
386 well as the contribution of LMWOS-C to SOM composition.

387 *4.3. Cytosol*

388 We calculated the ^{13}C enrichment of the cytosolic microbial C pool, extracted after
389 chloroform fumigation. The estimated turnover time of C in this pool was about 151 days

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

(Fig. 5). This value lies close to the previously reported range of 87–113 days, for the same pool for soils incubated for 98 days with ^{13}C glucose (Perelo and Munch, 2005), but were lower than MB C turnover time calculated using a conversion factor (2.22) - 82 days, for soils incubated for 60 days with ^{14}C glucose (Kouno et al., 2002). About 23% of the remaining glucose derived ^{13}C was still present in the cytosol after 50 days (Fig. 1), which is within the range found in previous studies (Grant et al., 1993). This agrees with the model of Nguyen and Guckert (2001) for the incorporation of glucose to the cytosol when applied at low input concentrations and its slow utilisation within the microbial cells.

4.4. Phospholipid fatty acids

4.4.1. Phospholipid fatty acid content and turnover

Phospholipid fatty acid C comprised 0.27% of the soil organic carbon (SOC). The ^{13}C incorporation into PLFAs, in case of constant PLFAs content during the experiment, reflects microbial activity under steady-state conditions (growth and death of microorganisms occur with the same rates) and processes of the exchange and replacement of existing PLFAs within living cells.

Few studies have estimated the C turnover time in PLFAs or the turnover time of PLFAs themselves in soil as very few options exist to estimate these parameters under steady-state conditions. The turnover time of ^{13}C-labelled PLFAs contained in dead microbial cells, was 2.7 days (Kindler et al., 2009). The PLFAs turnover times estimated in the field conditions using a C_3/C_4 vegetation change (Amelung et al., 2008; Glaser, 2005) or ^{14}C dating (Rethemeyer et al., 2005) were between 1 and 80 years. However, these approaches estimate the turnover time of C bound in PLFA, which can be much older than the PLFA molecules due to repeated C recycling before incorporation. In contrast, ^{13}C pulse labeling is

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

414 an approach that enables direct estimation of the turnover of freshly added C by the initial
415 incorporation peak. The approach used in the present study showed that the C turnover time
416 in PLFA is about 47 days (Fig. 2 and Fig. 5). Accordingly, if the decomposition after cell
417 death is about three days, the PLFA turnover time in living cells is about 44 days. This short
418 turnover time of PLFAs is significantly lower than the C turnover time in the cytosol (Fig. 2).
419 This is because the membrane is the interacting surface between the cell and the environment
420 and thus, frequent and rapid adaptations of its structure are crucial for active microorganisms.
421 In contrast, the extracted cytosolic pool includes C from both active and dormant
422 microorganisms (Blagodatskaya and Kuzyakov, 2013), and the latter can dilute the ^{13}C signal
423 incorporated into the active pool with non-labelled C, yielding a lower turnover of this pool.

424

425 *4.4.2. Contribution of microbial groups to glucose derived C utilisation*

426 Bacterial fatty acids dominated in the community structure measured by PLFA, and among
427 bacteria, fatty acids from G- bacteria were the most abundant (Table 1). Because we used
428 agricultural soil with pH close to neutral (6.6), the predominance of bacterial PLFAs was
429 expected. Filamentous microorganisms were represented mainly by actinomycetes and VAM,
430 which are also typical for agricultural soils (Dungait et al., 2011; McCarthy and Williams,
431 1992). We classified 16:1ω5 fatty acid as a biomarker for VAM (Olsson, 1999) and not for
432 G- bacteria, because: i) VAM are usually abundant in soils, where they form a symbiotic
433 relationship with up to 80% of land plants (Madan et al., 2002), and ii) 16:1ω5 behaved
434 similarly to fungi in terms of glucose derived C use (Fig. 3, top). For the precise
435 interpretation of 16:1ω5 as a VAM fatty acid, the simultaneous analysis of 16:1ω5 in
436 comparison to neutral lipids should be performed, otherwise, the relationship of 16:1ω5 to
437 VAM should be viewed with caution.

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

438 More glucose derived ^{13}C was incorporated into bacterial PLFAs (Fig. 3, top), than
439 filamentous microorganisms. This can be a consequence of low C loading rates (less than 4
440 mg C g-1 soil, see (Reischke et al., 2014), under which conditions the added C is utilized
441 primarily by bacterial communities, whereas at higher concentrations of applied substrate, the
442 dominance of fungi in substrate utilisation is observed (Reischke et al., 2014).

443 Total ^{13}C incorporation into gram-negative fatty acids was higher (taking both G-
444 groups together) compared to G+ bacterial PLFAs (Fig. 3, top), which might be due to: i) the
445 abundance of their fatty acids, which was higher (Table 1) or ii) glucose uptake activity,
446 which was higher for G- than G+ groups. In contrast, the ^{13}C enrichment (total ^{13}C
447 incorporation related to C in particular biomarkers) for G- bacterial PLFAs was not higher
448 than that for G+ (Fig. 3, bottom). Thus, the high total ^{13}C incorporation into G- bacterial
449 biomarkers can mainly corresponds to their high content in the soil, not to higher activity of
450 microbial groups. However, replacement of PLFAs C by glucose derived ^{13}C is only a proxy
451 of microbial activity and only partly confirm the real mechanisms. This clearly suggests that
452 the analysis of isotope data after labeling in general requires the calculation and combined
453 interpretation of both the total tracer C incorporation as well as the ^{13}C enrichment in the
454 investigated pool.

455 In contrast to our results, a higher incorporation of glucose derived ^{13}C into G+ than
456 G- PLFAs was observed in other studies (Dungait et al., 2011; Ziegler et al., 2005). However,
457 in these studies, much higher amounts of C were applied to the soil (15 µg C g^{-1} soil), which
458 stimulated the growth of G+ bacteria. In contrast, under steady-state conditions with low
459 glucose concentrations in soil, G- bacteria were the most competitive group for glucose
460 uptake (Fig. 3).

461 The ^{13}C enrichment of bacterial PLFAs decreased from day 3 to day 50, whereas ^{13}C
462 in fungal PLFAs increased (in the case of VAM) or stayed constant (Fig. 3, bottom). The

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

463 decrease in ^{13}C enrichment in bacterial fatty acids indicates a partial turnover of bacterial
464 lipid membranes, which is much faster than turnover in fungal membranes. This result is
465 consistent with the turnover time of bacterial biomass in soil (Baath, 1998), which is about 10
466 days, whereas fungal biomass turnover times range between 130–150 days (Rousk and Baath,
467 2007). Consequently, the increase in ^{13}C enrichment in fungal PLFAs at late sampling points
468 indicates that fungi consume the exudation products of bacteria or even dead bacterial
469 biomass (Zhang et al., 2013; Ziegler et al., 2005).

470

471 *4.5. Amino sugars*

472 *4.5.1. Amino sugar content and amino sugar C turnover in total and living microbial cell*
473 *walls*

474 Amino sugars represented the largest microbial pool investigated in this study (Table 1) and
475 comprised 3.7% of SOC. Chitin and peptidoglycan, the direct sources of the amino sugars,
476 comprise no more than 5% of cell biomass (Park and Uehara, 2008; Wallander et al., 2013).
477 Therefore, the high amount of amino sugars relative to PLFA can only be explained by their
478 high proportion in microbial residues/necromass (Glaser et al., 2004; Liang et al., 2008;
479 Glaser et al., 2004). Irrespective of the large pool size of the amino sugars, their incorporation
480 of glucose derived ^{13}C was the lowest compared to other compartments in living cells. The
481 total ^{13}C incorporation and enrichment of amino sugars increased from day 3 to day 50, in
482 contrast to cytosol and PLFA pools. Consequently, amino sugars have the slowest turnover in
483 soils, presumably even within living cells, for two reasons: 1) cell walls are polymers that
484 require a rather complex biosynthesis of the amino-sugar fibers, 2) cell-wall polymerization
485 occurs extracellularly (Lengeler et al., 1999) and 3) microorganisms do not need to

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

synthesize peptidoglycan unless they multiply. To calculate C turnover time in this pool, further long-term sampling points with ^{13}C-amino-sugar analysis are necessary.

The majority of amino sugars extracted after acid hydrolysis represent microbial necromass, which does not incorporate any glucose derived ^{13}C, but strongly dilutes the ^{13}C incorporated into the walls of living cells. To estimate the ^{13}C incorporation into amino sugars of living cells, we first calculated the amount of amino sugars in the living MB pool based on the fatty acids content. Assuming that PLFAs are present only in living biomass, and that the ratio of fatty acids to amino sugars in living biomass is about 0.23 (Lengeler et al., 1999), we estimated the amount of amino sugars in living MB to be 0.20 µmol g^{-1} soil fatty acids/0.23 = 0.87 µmol g^{-1} soil. The estimated percentage of amino sugars in living biomass from the total amino sugar pool was 0.87/7.70 (total AS (µmol g^{-1} soil))*100 = 11%. This estimate agrees with that of Amelung et al. (2001a) and Glaser et al. (2004), who reported that the amount of amino sugars in living biomass is one to two orders of magnitude lower than in the total amino-sugar pool. We calculated the ^{13}C enrichment in amino sugars for the first sampling point, assuming that all replaced C is still contained within living MB after three days of glucose C utilisation. Total tracer incorporation into amino sugars consisted of 0.00071 µmol glucose derived ^{13}C in amino sugars g^{-1} soil/0.87 (µmol amino sugars g^{-1} soil)*7 (mean amount of C atoms in amino sugars)*100 = 0.57% of the C pool. Comparison of these data with the ^{13}C enrichment into PLFAs and the cytosol allowed us to conclude that the replacement of the amino sugar C with glucose derived ^{13}C in living biomass is two-fold slower than the replacement in PLFAs, and faster than in the cytosolic pool. This reflects that microbial C turnover is a phenomenon that is not restricted to the death or growth of new cells, but that even within living cells, highly polymeric cell compounds, including cell walls, are constantly replaced and renewed (Park and Uehara, 2008).

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

511 *4.5.2. Contribution of bacterial and fungal cell walls to SOC*

512 Glucosamine was the dominant amino sugar in the soil, whereas muramic acid was the least
513 abundant (Table 1), which agrees with most literature data (Engelking et al., 2007; Glaser et
514 al., 2004). To conclude about the proportions of bacterial and fungal residues in the SOM, the
515 ratios of glucosamine/muramic acid and galactosamine/muramic acid (Glaser et al., 2004)
516 were calculated (Table 1). Based on the galactosamine/muramic acid ratio, bacteria were
517 dominant within the soil microbial community, whereas the glucosamine/muramic acid ratio
518 indicated that the fungal contribution was larger. This discrepancy is due to unknown sources
519 of glucosamine in the soil (Glaser et al., 2004), i.e. it originates from bacterial (Amelung et
520 al., 2001b) and fungal cell walls (Fernandez and Koide, 2012; Glaser et al., 2004; Park and
521 Uehara, 2008) as well as from actinomycetes, insect and invertebrate. Moreover, previous
522 studies have confirmed galactosamine to be the most characteristic amino sugar for fungal
523 residues in soil (Engelking et al., 2007) and thus, galactosamine/muramic acid ratio is more
524 useful for estimation the composition of microbial residues in SOM. The bacterial origin of
525 microbial residues in the soil is supported by: 1) the dominance of bacterial PLFA biomarkers
526 2) the environmental conditions of our the site, namely, long-term agricultural use, which
527 promotes the dominance of bacterial communities.

528 Three-fold more glucose derived ^{13}C was incorporated into glucosamine than into
529 galactosamine and muramic acid (Fig. 4, top). This correlates with the pool size and indicates
530 that glucosamine is the most dominant amino sugar not only in total amino sugars, but also
531 within the walls of living cells. The galactosamine/muramic acid ratio of the incorporated ^{13}C
532 was six, and consequently was significantly lower than the ratio calculated for the amount of
533 amino sugars (Table 1). This indicates that bacteria are more active in glucose derived ^{13}C
534 utilisation than fungi, a conclusion also supported by the ^{13}C-PLFA data (Fig. 3). Thus, even
535 if the composition of amino sugars does not allow a clear conclusion concerning living

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

536 microbial communities in soil, amino sugar analysis combined with ^{13}C labeling reveals the
537 activity of living microbial groups in terms of substrate utilisation.

538 The calculated ^{13}C enrichment was the highest in muramic acid (Fig. 4, bottom). This
539 is in agreement with the high ^{13}C enrichment of bacterial PLFAs compared to VAM and
540 fungi (Fig. 3). Due to differences in cell-wall architecture, G+ bacteria contain more muramic
541 acid (approximately four times) than G- bacteria (Lengeler et al., 1999), and thus make a
542 higher contribution to the ^{13}C enrichment of muramic acid.

543 The ^{13}C enrichment of glucosamine was two-fold lower than muramic acid (Fig. 4,
544 bottom). This confirms the hypothesis that glucosamine originates from bacterial as well as
545 fungal cell walls and consequently has a mixed enrichment between that of the fungal
546 galactosamine and the bacterial muramic acid.

547

548 **5. Conclusions**

549 Tracing the ^{13}C labelled glucose through cytosol, PLFAs and amino sugars is a prerequisite
550 for understanding the fate of organic substrates in soil. The highest ^{13}C enrichment, and thus
551 turnover of C, was found for the PLFA pool, corresponding to a turnover time of 47 days,
552 whereas the turnover was slower in the cytosol (150 days). These differences can be
553 attributed to 1) efficient C recycling in the cytosol, and 2) its heterogeneous composition,
554 which contains compounds with different turnover rates. The ^{13}C enrichment of amino sugars
555 was still increasing at the end of the experiment, reflecting the slowest C turnover within the
556 investigated pools and that most of this pool consisted of microbial residues and not living
557 biomass. An approximate calculation of ^{13}C enrichment of amino sugars in the living biomass
558 gave values 0.57% of pool size, which was still lower than for PLFAs. Thus, C turnover in

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

559 membrane components is faster than in cell wall components, even if only the portion of the
560 amino sugar pool in living biomass is considered.

561 Bacterial PLFAs dominated in the microbial community composition, and much
562 higher glucose derived ^{13}C was incorporated to bacterial than to fungal PLFAs too. This
563 agrees with prevailing role of bacteria in the utilisation of easily available organic substrates
564 that are present at low concentrations in soil. A slower turnover of filamentous and mainly
565 fungal biomass compared to bacteria was observed in the ^{13}C enrichment of the respective
566 PLFAs. Therefore, filamentous organisms might utilize the products of bacterial metabolism
567 and biomass, which is an important link in the soil food web.

568 The galactosamine/muramic acid ratio was between 12 and 19, indicating a
569 predominance of bacterial vs. fungal residues in SOM. The ratio of galactosamine/muramic
570 acid for incorporated ^{13}C confirmed that bacteria were more active in glucose utilisation than
571 fungi. The ^{13}C enrichment was the highest for muramic acid and the lowest for
572 galactosamine, demonstrating that the turnover of bacterial cell wall components is more
573 rapid than fungal.

574 Consequently, the combination of ^{13}C labeling with the subsequent analysis of several
575 microbial cell compartments and biomarkers is a unique approach to understanding C
576 partitioning within microbial cells and the microbial communities in soil. This knowledge is
577 not only crucial for assessing C fluxes and recycling in soil, but is also of special importance
578 concerning the contribution of C from microbial residues to SOM.

579

580 **Author contribution**

581 Y. Kuzyakov and B. Glaser designed the experiments and M. Dippold and A. Gunina carried
582 them out. A. Gunina prepared the manuscript with contributions from all co-authors.

583

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

584 **Data availability**

585 Underlying research data can be accessed by a request from the first author of paper.

586

587 **Acknowledgements**

588 This study was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG KU
589 1184 19/1 and INST 186/1006-1 FUGG). The authors are grateful to Stefanie Bösel, a
590 technical staff member of the Department of Soil Biochemistry, Institute of Agricultural and
591 Nutritional Science, Martin-Luther University Halle-Wittenberg for performing the bulk
592 isotope and amino sugars measurements. Thanks are extended to MolTer and DAAD, which
593 provided a fellowship for A. Gunina. We are very grateful to the Centre for Stable Isotope
594 Research and Analysis (KOSI) of Göttingen University for the $\delta^{13}C$ measurements.

595

596

597

598

599

600

601

602

603

604

605

606

607

608

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

References

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., and Bol, R.: Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter, in: Advances in Agronomy, Vol 100, Sparks, D. L. (Ed.), Advances in Agronomy, 155–250, 2008.

Amelung, W., Kimble, J. M., Samson-Liebig, S., and Follett, R. F.: Restoration of microbial residues in soils of the Conservation Reserve Program, Soil Science Society of America Journal, 65, 1704–1709, 2001a.

Amelung, W., Miltner, A., Zhang, X., and Zech, W.: Fate of microbial residues during litter decomposition as affected by minerals, Soil Science, 166, 598–606, doi:10.1097/00010694-200109000-00003, 2001b.

Apostel, C., Dippold, M. A., Glaser, B., and Kuzyakov, Y.: Biochemical pathways of amino acids in soil: Assessment by position-specific labeling and ^{13}C-PLFA analysis, Soil Biology & Biochemistry, 67, 31–40, 2013.

Baath, E.: Growth rates of bacterial communities in soils at varying pH: A comparison of the thymidine and leucine incorporation techniques, Microbial Ecology, 36, 316–327, doi:10.1007/s002489900118, 1998.

Bai, Z., Bode, S., Huygens, D., Zhang, X., and Boeckx, P.: Kinetics of amino sugar formation from organic residues of different quality, Soil Biology & Biochemistry, 57, 814–821, doi:10.1016/j.soilbio.2012.08.006, 2013.

Blagodatskaya, E. and Kuzyakov, Y.: Active microorganisms in soil: critical review of estimation criteria and approaches, Soil Biology & Biochemistry, 67, 192–211, 2013.

Boddy, E., Hill, P. W., Farrar, J., and Jones, D. L.: Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils, Soil Biology & Biochemistry, 39, 827–835, doi:10.1016/j.soilbio.2006.09.030, 2007.

Brant, J. B., Sulzman, E. W., and Myrold, D. D.: Microbial community utilization of added carbon substrates in response to long-term carbon input manipulation, Soil Biology & Biochemistry, 38, 2219–2232, doi:10.1016/j.soilbio.2006.01.022, 2006.

Bremer, E. and Kuikman, P.: Microbial utilization of ^{14}C-U-glucose in soil is affected by the amount and timing of glucose additions, Soil Biology & Biochemistry, 26, 511–517, doi:10.1016/0038-0717(94)90184-8, 1994.

Derrien, D., Marol, C., and Balesdent, J.: The dynamics of neutral sugars in the rhizosphere of wheat. An approach by C-13 pulse-labelling and GC/C/IRMS, Plant and Soil, 267, 243–253, doi:10.1007/s11104-005-5348-8, 2004.

Dippold, M. A., Boesel, S., Gunina, A., Kuzyakov, Y., and Glaser, B.: Improved delta C-13 analysis of amino sugars in soil by ion chromatography-oxidation-isotope ratio mass spectrometry, Rapid Communications in Mass Spectrometry, 28, 569–576, doi:10.1002/rcm.6814, 2014.

Dungait, J. A. J., Kemmitt, S. J., Michallon, L., Guo, S., Wen, Q., Brookes, P. C., and Evershed, R. P.: Variable responses of the soil microbial biomass to trace concentrations of ^{13}C-labelled glucose, using 13C-PLFA analysis, European Journal of Soil Science, 62, 117–126, doi:10.1111/j.1365-2389.2010.01321.x, 2011.

Engelking, B., Flessa, H., and Joergensen, R. G.: Shifts in amino sugar and ergosterol contents after addition of sucrose and cellulose to soil, Soil Biology & Biochemistry, 39, 2111–2118, doi:10.1016/j.soilbio.2007.03.020, 2007.

Fernandez, C. W. and Koide, R. T.: The role of chitin in the decomposition of ectomycorrhizal fungal litter, Ecology, 93, 24–28, 2012.

Fischer, H., Meyer, A., Fischer, K., and Kuzyakov, Y.: Carbohydrate and amino acid composition of dissolved organic matter leached from soil, Soil Biology & Biochemistry, 39, 2926–2935, doi:10.1016/j.soilbio.2007.06.014, 2007.

659 Frostegard, A., Tunlid, A., and Baath, E.: Microbial biomass measured as total lipid
660 phosphate in soils of different organic content, Journal of Microbiological Methods, 14,
661 151–163, doi:10.1016/0167-7012(91)90018-l, 1991.
662 Frostegard, A., Tunlid, A., and Baath, E.: Use and misuse of PLFA measurements in soils,
663 Soil Biology & Biochemistry, 43, 1621–1625, doi:10.1016/j.soilbio.2010.11.021, 2011.
664 Gearing, P. J., Gearing, J. N., Maughan, J. T., and Oviatt, C. A.: Isotopic Distribution of
665 Carbon from Sewage Sludge and Eutrophication in the Sediments and Food Web of
666 Estuarine Ecosystems, Environmental Science & Technology, 25, 295–301, 1991.
667 Glanville, H., Rousk, J., Golyshin, P., and Jones, D. L.: Mineralization of low molecular
668 weight carbon substrates in soil solution under laboratory and field conditions, Soil
669 Biology & Biochemistry, 48, 88–95, doi:10.1016/j.soilbio.2012.01.015, 2012.
670 Glaser: Compound-specific stable-isotope ($d^{13}C$) analysis in soil science, 2005.
671 Glaser, B. and Amelung, W.: Determination of ^{13}C natural abundance of amino acid
672 enantiomers in soil: methodological considerations and first results, Rapid
673 Communications in Mass Spectrometry, 16, 891–898, doi:10.1002/rcm.650, 2002.
674 Glaser, B. and Gross, S.: Compound-specificδ13C analysis of individual amino sugars - a
675 tool to quantify timing and amount of soil microbial residue stabilization, Rapid
676 Commun. Mass Spectrom., 19, 1409–1416, doi:10.1002/rcm.1913, 2005.
677 Glaser, B., Millar, N., and Blum, H.: Sequestration and turnover of bacterial- and fungal-
678 derived carbon in a temperate grassland soil under long-term elevated atmospheric
679 pCO(2), Global Change Biology, 12, 1521–1531, doi:10.1111/j.1365-2486.2006.01186.x,
680 2006.
681 Glaser, B., Turrion, M. B., and Alef, K.: Amino sugars and muramic acid - biomarkers for
682 soil microbial community structure analysis, Soil Biology & Biochemistry, 36, 399–407,
683 doi:10.1016/j.soilbio.2003.10.013, 2004a.
684 Grant, R. F., Juma, N. G., and McGill, W. B.: Simulation of carbon and nitrogen
685 transformations in soils. II. Microbial biomass and metabolic products, Soil Biology &
686 Biochemistry, 25, 1331–1338, doi:10.1016/0038-0717(93)90047-f, 1993.
687 Gregorich, E. G., Voroney, R. P., and Kachanoski, R. G.: Turnover of carbon through the
688 microbial biomass in soil with different textures, Soil Biology & Biochemistry, 23, 799–
689 805, doi:10.1016/0038-0717(91)90152-a, 1991.
690 Gunina, A., Dippold, M., Glaser, B., and Kuzyakov, Y.: Fate of low molecular weight
691 organic substances in an arable soil: From microbial uptake to utilisation and stabilisation,
692 Soil Biology & Biochemistry, 77, 304–313, 2014.
693 Gunina, A. and Kuzyakov, Y.: Sugars in soil and sweets for microorganisms: Review of
694 origin, content, composition and fate, Soil Biology and Biochemistry,
695 doi:10.1016/j.soilbio.2015.07.021, 2015.
696 Indorf, C., Stamm, F., Dyckmans, J., and Joergensen, R. G.: Determination of saprotrophic
697 fungi turnover in different substrates by glucosamine-specific delta C-13 liquid
698 chromatography/isotope ratio mass spectrometry, Fungal Ecology, 5, 694–701,
699 doi:10.1016/j.funeco.2012.07.001, 2012.
700 Jenkinson, D. S. and Ladd, J. N.: Microbial biomass in soil: measurement and turnover, Soil
701 Biochemistry, Marcel Dekker, New York, 415–471, 1981.
702 Kindler, R., Miltner, A., Richnow, H.-H., and Kaestner, M.: Fate of gram-negative bacterial
703 biomass in soil - mineralization and contribution to SOM, Soil Biology & Biochemistry,
704 38, 2860–2870, doi:10.1016/j.soilbio.2006.04.047, 2006.
705 Kindler, R., Miltner, A., Thullner, M., Richnow, H.-H., and Kaestner, M.: Fate of bacterial
706 biomass derived fatty acids in soil and their contribution to soil organic matter, Organic
707 Geochemistry, 40, 29–37, doi:10.1016/j.orggeochem.2008.09.005, 2009.
708 Knapp, D.: Handbook of Analytical Derivatization Reagents, Wiley, New York, 1979.

709 Kouno, K., Wu, J., and Brookes, P. C.: Turnover of biomass C and P in soil following
710 incorporation of glucose or ryegrass, Soil Biology & Biochemistry, 34, 617–622,
711 doi:10.1016/s0038-0717(01)00218-8, 2002.
712 Kuzyakov, Y.: How to link soil C pools with CO_2 fluxes?, Biogeosciences, 8, 1523–1537,
713 doi:10.5194/bg-8-1523-2011, 2011.
714 Lengeler, J. W., Drews, G., and Schlegel, H. G.: Biology of the prokaryotes, Georg Thieme
715 Verlag, 955, 1999.
716 Liang, C., Fujinuma, R., and Balser, T. C.: Comparing PLFA and amino sugars for microbial
717 analysis in an Upper Michigan old growth forest, Soil Biology & Biochemistry, 40, 2063–
718 2065, doi:10.1016/j.soilbio.2008.01.022, 2008.
719 Lutzow, M. v., Koegel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G.,
720 Marschner, B., and Flessa, H.: Stabilization of organic matter in temperate soils:
721 mechanisms and their relevance under different soil conditions - a review, European
722 Journal of Soil Science, 57, 426–445, doi:10.1111/j.1365-2389.2006.00809.x, 2006.
723 Madan, R., Pankhurst, C., Hawke, B., and Smith, S.: Use of fatty acids for identification of
724 AM fungi and estimation of the biomass of AM spores in soil, Soil Biology &
725 Biochemistry, 34, 125–128, doi:10.1016/s0038-0717(01)00151-1, 2002.
726 Malik, A., Blagodatskaya, E., and Gleixner, G.: Soil microbial carbon turnover decreases
727 with increasing molecular size, Soil Biology & Biochemistry, 62, 115–118,
728 doi:10.1016/j.soilbio.2013.02.022, 2013.
729 McCarthy, A. J. and Williams, S. T.: Actinomycetes as agents of biodegradation in the
730 environment - a review, Gene, 115, 189–192, doi:10.1016/0378-1119(92)90558-7, 1992.
731 Moore, J. C., McCann, K., and Ruiter, P. C. de: Modeling trophic pathways, nutrient cycling,
732 and dynamic stability in soils, Pedobiologia, 49, 499–510,
733 doi:10.1016/j.pedobi.2005.05.008, 2005.
734 Nguyen, C. and Guckert, A.: Short-term utilisation of C-14- U glucose by soil
735 microorganisms in relation to carbon availability, Soil Biology & Biochemistry, 33, 53–
736 60, doi:10.1016/s0038-0717(00)00114-0, 2001.
737 Olsson, P. A.: Signature fatty acids provide tools for determination of the distribution and
738 interactions of mycorrhizal fungi in soil, Fems Microbiology Ecology, 29, 303–310, 1999.
739 Park, J. T. and Uehara, T.: How bacteria consume their own exoskeletons (Turnover and
740 recycling of cell wall peptidoglycan), Microbiology and Molecular Biology Reviews, 72,
741 211–227, doi:10.1128/mmbr.00027-07, 2008.
742 Parton, W. J., Schimel, D. S., Cole, C. V., and Ojima, D. S.: Analysis of factors controlling
743 soil organic matter levels in Great Plains grasslands, Soil Sci. Soc. Am. J., 51, 1173–
744 1179, 1987.
745 Perelo, L. W. and Munch, J. C.: Microbial immobilisation and turnover of (13)C labelled
746 substrates in two arable soils under field and laboratory conditions, Soil Biology &
747 Biochemistry, 37, 2263–2272, doi:10.1016/j.soilbio.2005.02.039, 2005.
748 Reischke, S., Rousk, J., and Bååth, E.: The effects of glucose loading rates on bacterial and
749 fungal growth in soil, Soil Biology & Biochemistry, 70, 88–95, 2014.
750 Rethemeyer, J.: Age heterogeneity of soil organic matter, 2004.
751 Rethemeyer, J., Kramer, C., Gleixner, G., John, B., Yamashita, T., Flessa, H., Andersen, N.,
752 Nadeau, M. J., and Grootes, P. M.: Transformation of organic matter in agricultural soils:
753 radiocarbon concentration versus soil depth, Geoderma, 128, 94–105,
754 doi:10.1016/j.geoderma.2004.12.017, 2005.
755 Rousk, J. and Baath, E.: Fungal biomass production and turnover in soil estimated using the
756 acetate-in-ergosterol technique, Soil Biology & Biochemistry, 39, 2173–2177,
757 doi:10.1016/j.soilbio.2007.03.023, 2007.

758 Ruess, L., Schutz, K., Haubert, D., Haggblom, M. M., Kandeler, E., and Scheu, S.:
759 Application of lipid analysis to understand trophic interactions in soil, Ecology, 86, 2075–
760 2082, doi:10.1890/04-1399, 2005.
761 Schimel, J. P. and Weintraub, M. N.: The implications of exoenzyme activity on microbial
762 carbon and nitrogen limitation in soil: a theoretical model, Soil Biology & Biochemistry,
763 35, 549–563, 2003.
764 Schmidt, S. K., Costello, E. K., Nemergut, D. R., Cleveland, C. C., Reed, S. C., Weintraub,
765 M. N., Meyer, A. F., and Martin, A. M.: Biogeochemical consequences of rapid microbial
766 turnover and seasonal succession in soil, Ecology, 88, 1379–1385, doi:10.1890/06-0164,
767 2007.
768 Schneckenberger, K., Demin, D., Stahr, K., and Kuzyakov, Y.: Microbial utilization and
769 mineralization of (14)C glucose added in six orders of concentration to soil, Soil Biology
770 & Biochemistry, 40, 1981–1988, doi:10.1016/j.soilbio.2008.02.020, 2008.
771 Wagner GH: Significance of microbial tissue to soil organic matter. In: Isotopes and radiation
772 in soil organic matter studies, FAO/IAEA, Vienna, 197–205, 1968.
773 Wallander, H., Ekblad, A., Godbold, D. L., Johnson, D., Bahr, A., Baldrian, P., Bjork, R. G.,
774 Kieliszewska-Rokicka, B., Kjoller, R., Kraigher, H., Plassard, C., and Rudawska, M.:
775 Evaluation of methods to estimate production, biomass and turnover of ectomycorrhizal
776 mycelium in forests soils - A review, Soil Biology & Biochemistry, 57, 1034–1047,
777 doi:10.1016/j.soilbio.2012.08.027, 2013.
778 Waring, B. G., Averill, C., Hawkes, C. V., and Holyoak, M.: Differences in fungal and
779 bacterial physiology alter soil carbon and nitrogen cycling: Insights from meta-analysis
780 and theoretical models, Ecol Lett, 16, 887–894, doi:10.1111/ele.12125, 2013.
781 Wu, J., Brookes, P. C., and Jenkinson, D. S.: Formation and destruction of microbial biomass
782 during the decomposition of glucose and ryegrass in soil, Soil Biology & Biochemistry,
783 25, 1435–1441, doi:10.1016/0038-0717(93)90058-j, 1993.
784 Wu, J., Joergensen, R. G., Pommerening, B., Chaussod, R., and Brookes, P. C.: Measurement
785 of soil microbial biomass C by fumigation extraction - an automated procedure, Soil
786 Biology & Biochemistry, 22, 1167–1169, doi:10.1016/0038-0717(90)90046-3, 1990.
787 Zelles, L.: Phospholipid fatty acid profiles in selected members of soil microbial
788 communities, Chemosphere, 35, 275–294, doi:10.1016/s0045-6535(97)00155-0, 1997.
789 Zelles, L., Palojarvi, A., Kandeler, E., VonLutzow, M., Winter, K., and Bai, Q. Y.: Changes
790 in soil microbial properties and phospholipid fatty acid fractions after chloroform
791 fumigation, Soil Biology & Biochemistry, 29, 1325–1336, doi:10.1016/s0038-
792 0717(97)00062-x, 1997.
793 Zhang, H., Ding, W., Yu, H., and He, X.: Carbon uptake by a microbial community during
794 30-day treatment with C-13-glucose of a sandy loam soil fertilized for 20 years with NPK
795 or compost as determined by a GC-C-IRMS analysis of phospholipid fatty acids, Soil
796 Biology & Biochemistry, 57, 228–236, doi:10.1016/j.soilbio.2012.08.024, 2013.
797 Zhang, X. D. and Amelung, W.: Gas chromatographic determination of muramic acid,
798 glucosamine, mannosamine, and galactosamine in soils, Soil Biology & Biochemistry, 28,
799 1201–1206, doi:10.1016/0038-0717(96)00117-4, 1996.
800 Ziegler, S. E., White, P. M., Wolf, D. C., and Thoma, G. J.: Tracking the fate and recycling
801 of C-13-labeled glucose in soil, Soil Science, 170, 767–778,
802 doi:10.1097/01.ss.0000190511.64169.9c, 2005.
803

804

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

805 **Table 1** Amount of microbial biomass compartments, their C content, PLFA content of
806 microbial groups and composition of microbial residues in investigated soil. G-1 and G-2 are
807 gram-negative group one and two, respectively; G+1 and G+2 are gram positive group one
808 and two, respectively; Ac – actinomycetes; VAM - vesicular arbuscular mycorrhiza fungi.
809 Data present mean of three time points ± SE

Compartment	mg component C kg^{-1} soil	mg kg^{-1} soil	Ratio
Cytosol	201.0 ± 7.1	-	
Phospholipid fatty acids	39.4 ± 4.7	51.9 ± 6.2	
Specific phospholipid fatty acids			
G-1	8.9±3.6	11.6 ± 4.6	
G-2	5.6±0.8	7.4 ± 1.1	
G+1	5.9±1.2	7.9 ± 1.6	
G+2	0.7±0.3	1.0 ± 0.4	
Ac	2.3±0.7	3.0 ± 1.0	
VAM	1.7±0.3	2.2 ± 0.3	
Fungi	1.0±0.2	1.3 ± 0.2	
Bacteria/Fungi			6 - 8.5
Amino sugars	560.7 ± 68.2	1393.8 ± 170.0	
Glucosamine	460.7±79.3	1146.5 ± 197.3	
Galactosamine	90.9±11.3	226.3 ± 28.2	
Muramic acid	9.1±1.8	21.1 ± 4.1	
Glucosamine/muramic acid			17 - 55
Glucosamine/muramic acid (literature data for pure cultures*)		Bacteria Fungi	5.3 271
Galactosamine/muramic acid			12 - 19
Galactosamine/muramic acid literature data for pure cultures*)		Bacteria Fungi	2.8 59

810 *Data are taken from Glaser et al. (2004).
811
812
813
814
815
816
817

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

818 **Table captions**

819 **Table 1** Amount of microbial biomass compartments, their C content, content of microbial
820 groups and composition of microbial residues in investigated soil. G-1 and G-2 are gram-
821 negative group one and two, respectively; G+1 and G+2 are gram positive group one and
822 two, respectively; Ac – actinomycetes; VAM - vesicular arbuscular mycorrhiza fungi.

823

824 **Figure captions**

825 **Fig. 01** Partitioning of glucose derived ^{13}C in SOM presented as the total ^{13}C incorporation
826 between the following pools: non-specified SOM, cytosolic, PLFAs and amino sugars. Small
827 letters reflect differences between the sampling points for the distinct pool. Data present
828 mean (n=4) and bars present standard errors.

829

830 **Fig. 02** ^{13}C enrichment in the cytosolic, PLFA and amino-sugar cell pools as well as
831 functions to calculate the C turnover times in these microbial cell pools. The left y-axis
832 represents the PLFA pool, the first right y-axis, the cytosolic and the second y-axis, the
833 amino-sugar pool. Data present mean (n=4) and bars present standard errors.

834

835 **Fig. 03** Total incorporation of glucose derived ^{13}C (top) and ^{13}C enrichment (bottom) of the
836 microbial PLFAs. Note that the values for VAM and fungi are scaled-up 10 times (secondary
837 Y axis) compared to those of other groups (Y axis at the left). Data present mean (n=4) and
838 bars present standard errors. Small letters reflect differences between the microbial groups for
839 total ^{13}C incorporation and ^{13}C enrichment from glucose; letters a-d are for day three, l-o are
840 for day 10, x-z are for day 50.

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

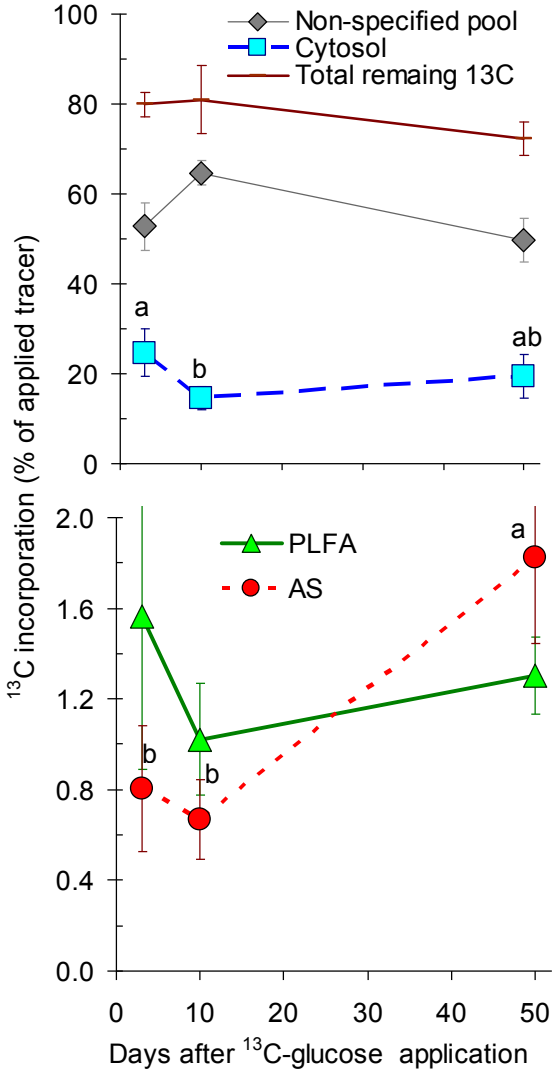
841 **Fig. 04** Total incorporation of glucose derived ^{13}C (top) and ^{13}C enrichment (bottom) of
842 amino sugars and muramic acid. Letters reflect significant differences in the total
843 incorporation and ^{13}C enrichment from glucose into amino sugars on a particular day; letters
844 a-b are for day three, l-m are for day 10, x-y are for day 50. No significant differences were
845 observed between the three sampling days. Data present mean (n=4) and bars present
846 standard errors.

847 **Fig. 05** Dynamic relationship of microbial utilization of glucose and turnover of cytosol, cell
848 membrane and cell wall components.

849
850
851
852
853
854
855
856
857
858
859
860
861
862
863
864
865
866
867
868
869
870
871
872
873
874
875
876
877
878
879
880
881

Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

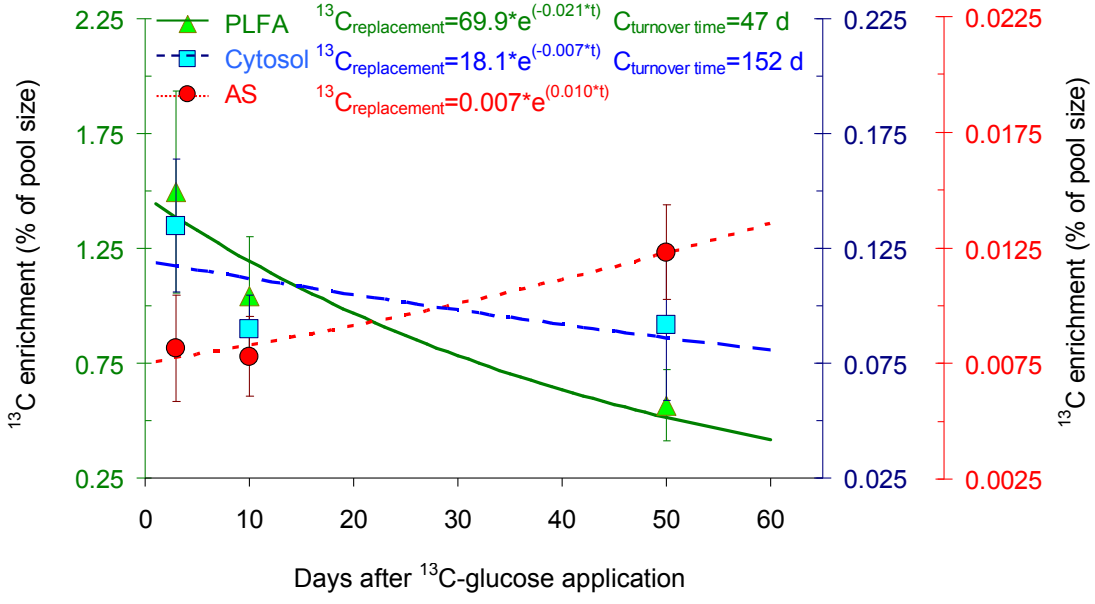
882 Figure 01.
883



Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

884 Figure 02.

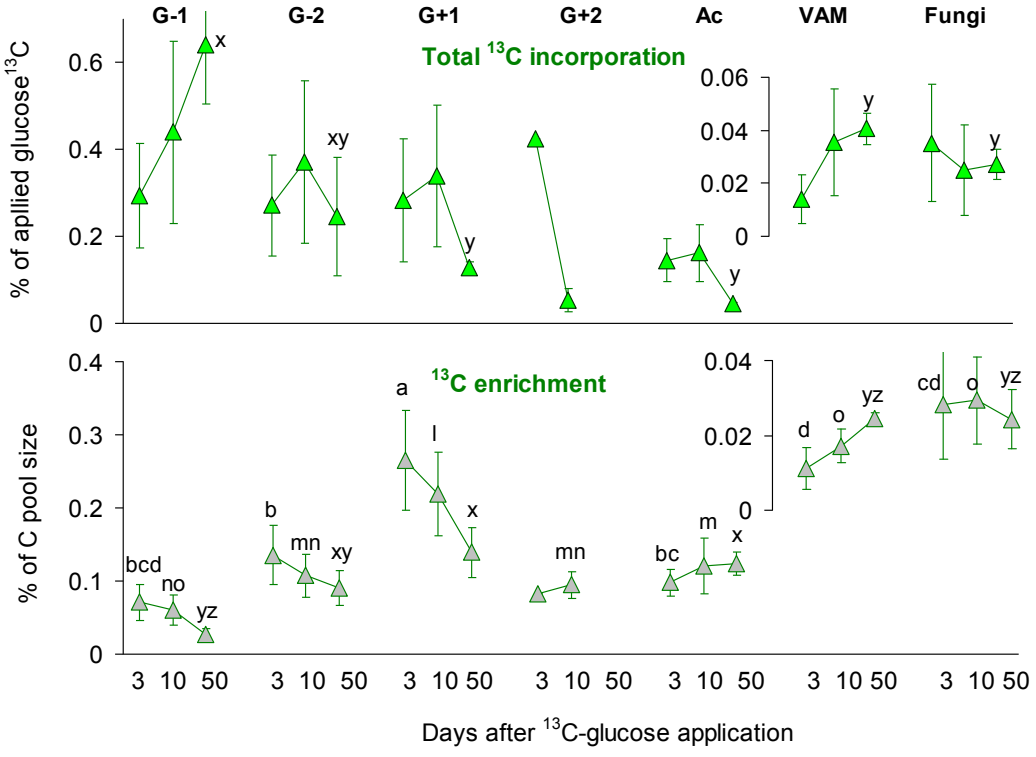
885



Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

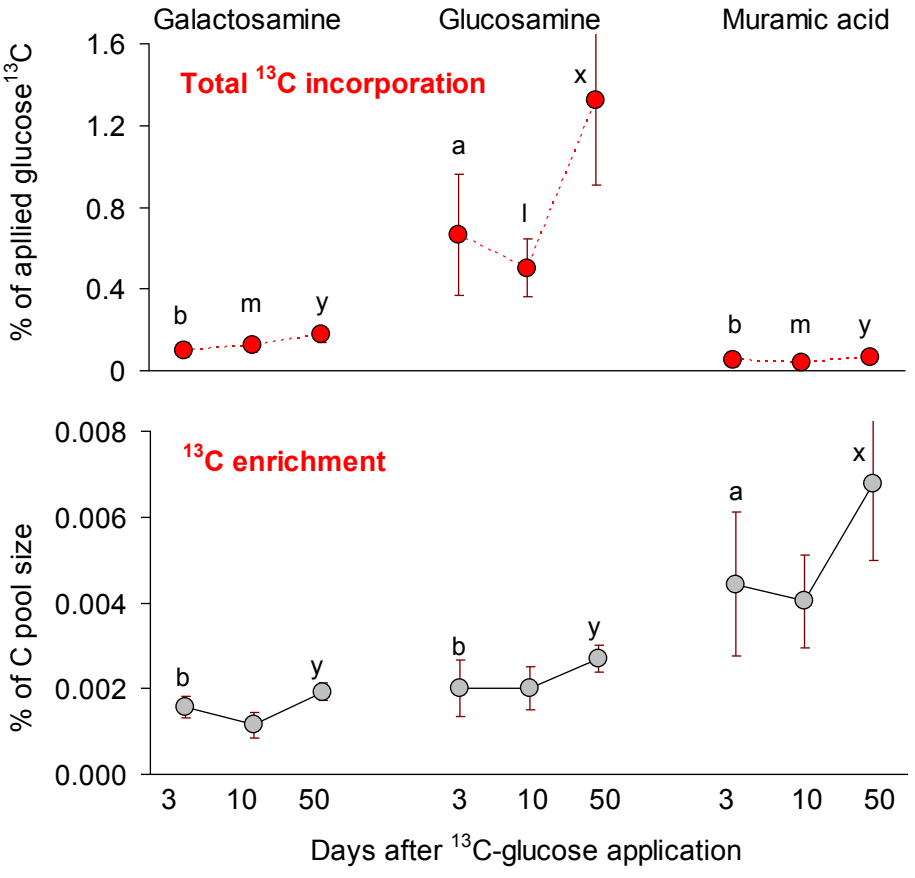
886 Figure 03.

887



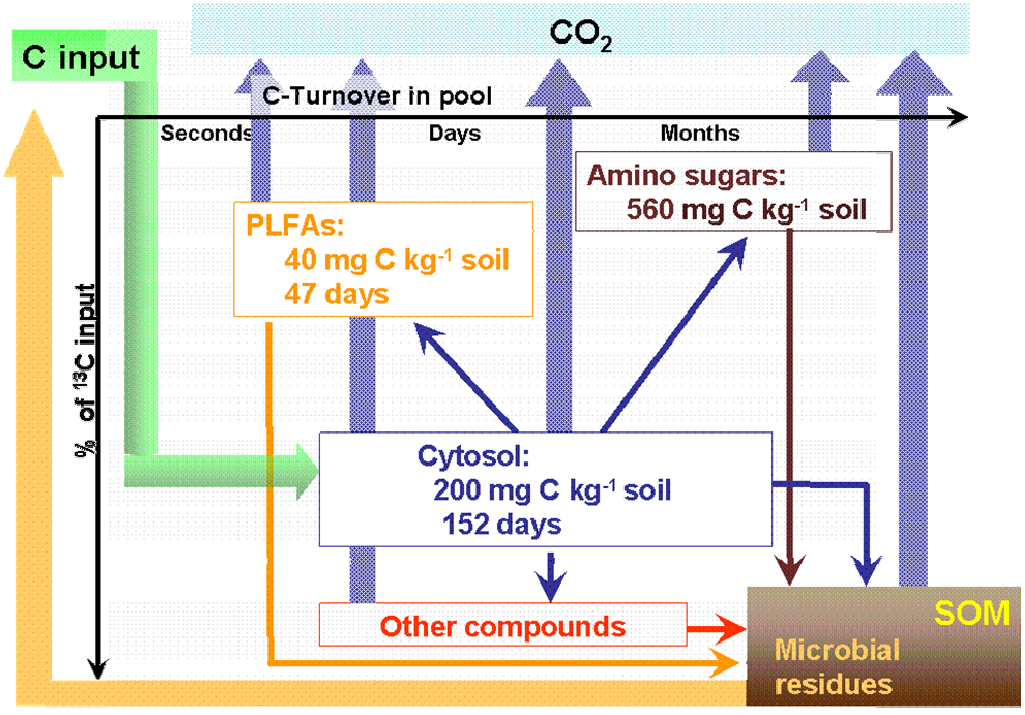
Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

888 Figure 04.



Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Manuscript under review for journal Biogeosciences
Published: 7 June 2016

889 Figure 05.



890
891

892

893

894