Определение стиля: Знак Знак: Шрифт: не курсив, интервал После: 6 пт

Revision of the Ms. No. Biogeosciences Discuss., doi:10.5194/bg-2016-214, 2016
Glucose C turnover in cell compartments and microbial groups in soil

Dear Prof. Pendall,

we are very thankful to the reviewers for their helpful suggestions and recommendations. We carefully improved the ms according to their comments and incorporated nearly all their suggestions.
Please find below the response to the reviewers' comments (**in black**) and the improved ms.

We hope that the ms fulfill the requirements of Biogeosciences.

With kind regards,
Anna Gunina and co-authors.

Reviewer 1.
I have revised ms CARBON TURNOVER IN CELL COMPARTMENTS AND MICROBIAL GROUPS IN SOIL by Gunina et al. To reveal the contribution of particular microbial groups to C utilization and C turnover within the microbial cells, the fate of 13Clabeled glucose was studied under field conditions in this work. The 13C was traced in cytosolic substances, amino sugars and phospholipid fatty acids (PLFA) at intervals of 3, 10 and 50 days after glucose addition. The strong and positive site of this manuscript is the combination of 13C labeling with the subsequent analysis of several microbial cell compartments and biomarkers as a unique approach to understanding C partitioning within microbial cells and the microbial communities in soil. This knowledge is crucial for both assessing C fluxes and their recycling in soil, and studying the contribution of C from microbial residues to SOM. There are some weaknesses of the manuscript, but they do not affect too much on the paper quality. Below I have included general and specific comments regarding all sections, and after all I recommend to authors revise, clarify and substitute some information through the whole text. To conclude, I think
present manuscript may be published in Biogeoscience journal.

Thank you very much for the evaluation of the ms!

General comments
Materials and methods: There is no information on bulk density of the soil in the paper. Provide this information, as it is not clear how you calculated soil mass in cylinder

The bulk density is 1.36 g cm-3. This was be added into materials and method section.

Check all links to figures, equations and tables and present them whether in full text or shortened form (fig, eq., etc), but the same through the whole manuscript.

Thank you for the comments. Corrected.

Specific comments

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L 180 – 183 I recommend to change “due to “in “Due to fumigation–extraction technique allows. . ...” for “As fumigation–extraction technique allows. . ..”

Was corrected: " As fumigation–extraction technique allows to obtain not only soluble components, but also cell organelles and cell particles, we named pool of C in fumigated extracts as cytosol only for simplification of terminology."

L 186 Revise sentence: In After organic C concentration were measured → After organic C concentrations were measured OR After organic C concentration was measured

Corrected " After organic C concentrations were measured, the K_2SO_4 extracts were freeze-dried and the $\delta^{13}C$ values....".

L 295 Clarify if it was 50th day of experiment or after 50 days in the sentence “13C in amino sugars increased two fold during the 50 day experiment (p<0.05)”

Corrected: " In contrast, ^{13}C in amino sugars increased two fold on the 50th day of experiment (p<0.05)".

L 301-302 Paraphrase the sentence, as it is difficult to read: “The enrichment was the highest in PLFAs (Fig. 2) and was 5–8 times lower in the cytosolic pool. The 13C enrichment in amino sugars was the lowest (Fig. 2) “

Corrected: "The pool enrichment was the highest for PLFAs and the lowest for amino sugars (Fig. 2)".

L 427- 429 Substitute “we used agricultural soil” on “we conduct experiment on agricultural soil”. In your paper you wrote about field experiment, not incubation one with agricultural soil. It is not correct “to use” soil in this situation.

Thank you for the comment, it was corrected: "Because we conduct experiment on agricultural soil with pH close to neutral (6.6), the predominance of bacterial PLFAs was expected".

L 450-451 Revise sentence “However, replacement of PLFAs C by glucose derived 13C is only a proxy of microbial activity and only partly confirms the real mechanisms.”

Corrected: "However, replacement of PLFAs C by glucose derived ^{13}C is only a proxy of microbial activity and can only partly estimate the real activity of microbial groups".

L 486-487 What long-term sampling points mean? Maybe, you suggest conducting long-term experiment for turnover time calculation? Or add sampling points to existing experiment?

Corrected: "To calculate C turnover time in this pool, conducting of long-term experiments is necessary".

L 526- 527 Substitute “our the site” on “experimental site or site” in “the environmental conditions of our the site, namely, long-term agricultural use. . ..”

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Corrected: "...the environmental conditions of the site, namely, long-term agricultural use, which promotes the dominance of bacterial communities".

Tables
L 809 It will be better to write “Data present mean of three replicates” instead of “Data present mean of three time points”.

Corrected: "Data present mean of three time points (with four replications for each time point) ± SE".

Figures
L 887 It is difficult to read graphs for Ac. Expand the space between Ac and second scale for VAM and Fungi.

Was corrected.

Reviewer 2.
Anonymous Referee #2
Received and published: 26 July 2016
Contributing to the understanding the C turnover of different cell compartments is certainly a valuable goal. This manuscript reports the findings of a 3-50 day incubation experiment where 13C from glucose is followed into the cytosol, PLFAs and amino sugars. They set out to test three hypotheses: (1) turnover times increase in the order cytosol>PLFA>amino sugars; (2) incorporation of 13C is faster for bacterial than fungal markers; (3) “due to amino sugars have long turnover times and are mainly dominated in microbial necromass, all incorportated 13C can be related only to living biomass and allow estimate percent of replaced C in amino sugars of living organisms. I am concerned about the limitations brought by sampling intensity and analysis of the data and mostly that the findings that were possible with this study don’t really allow for testing the hypotheses presented. Probably as a result. A lot of what’s presented in the Discussion is not critical or essential and it’s either general, repetitive or tangential.

Thank you for the study evaluations.

1. Hypothesis 1 could not really be tested given the duration and intervals of sampling during the experiment. Glucose gets processed, incorporated, lost and recycled into PLFA already in the first two days (e.g. Ziegler 2005) and this can vary with soil and environmental conditions. Starting measurements after 3 days leaves us without any information of when the peak of uptake took place (thus when time zero for decomposition started) and when recycling started which would matter for trying to estimating turnover. Then on the other end, 50 day was not sufficient time for the aminosugars to finish building up and start decomposing, as the authors discuss. Perhaps the data can be used to answer a different question. From Figure 2, we don’t know how good the model fits were (it would be important given there were three points and large error bars).

Thank you for the comment. The first reason, that time 0 was not taken into account, because uptake of glucose by microorganisms from soil solution can be slower for the field

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conditions than in lab observations (minute range (Hill et al., 2008). Thus, part of the added glucose still can be in soil solution 1 day after addition and incorporation of ^{13}C into cytosol will still increase. Thus, it would not be possible to calculate C turnover time in cytosol pool. The second reason is: even if glucose is completely taken by microorganisms, the glucose-derived C incorporation into other pools is delayed (glucose C can be stored in cytosol, and only later used for biosynthesis), and thus, it would not be possible to have the t0 for all cellular pools. The third reason is that our experiment was focused on the 2nd phase of glucose utilization (using in biosynthesis) and not on the first (uptake phase).

Figure 2: We agree that we do not have big amount of points, but curve fitting was done for 4 replications for each time point. Due to calculation of turnover time is related to the big uncertainty, not a lot of conclusions were made based on that parameter. Even there are some uncertainties with calculating the C turnover times, the increase or decrease of ^{13}C incorporation into the cellular pools with time can evidence about slow or fast turnover of particular pools. Moreover our experiment was done in the field and, even the increasing the amount of points would not help to reduce the errors, because much larger variations can be expected in the field conditions compare to lab experiments, where soil is homogenized before the experiment.

2. Hypothesis 2 is about differential incorporation by bacteria and fungi (who incorporates it faster). Again, missing the first three day is pretty critical (Ziegler 2005 clearly shows this). There are already many experiments that have assessed "initial" incorportation of 13C into biomarker lipids and we wouldn't then need 50 days incubation for this. Also, I am surprised that there was no effect of time on the composition of the PLFA as 50 days is quite a long time for microbes and PLFA profiles tend to be more sensitive to time than any other driver, and, C depletion in 50 days of an incubation would be substantial.

Thank you for the comment. Yes, we agree that the time is very important in the labeling experiments, especially if the labile substances are applied. However, our experiment was done in the field (with 1.5 kg of soil and changes of the environmental condition between day and night), and microorganisms can take up glucose much slower compare to lab experiments. In this case measurement of ^{13}C in the PLFAs on the 1st or 2nd day will probably not show the highest incorporation. That is why we did not make the 1st point 1 day after ^{13}C incorporation. Concerning the initial incorporation: most of the experiments were done in the laboratory, whereas our experiment was done in the field conditions, where processes go much slower. The data on total remaining ^{13}C after 50 days of experiment are clearly show this: around 70% of ^{13}C derived glucose remained, showing slow rates of processes in the field experiment. Moreover, we were focused on the 2nd phase of glucose utilization and not on the first.

The structure of PLFAs stayed quite stable during the field experiment because of the following: i) soil was plowed two week before the experiment and microorganisms were already adopted to the limiting C conditions and ii) low concentration of glucose was applied, which did not promote microbial growth or changes of microbial community structure.

3. Hypothesis 3 is hard to follow. I tried but was not able to understand it.

Thank you for the comment. We have deleted hypothesis 3.

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4. In the Introduction, the paragraph comprising Lines 96-108 is a very convoluted and hard to follow, however, it refers to the main rational for carrying out this study.

This paragraphs was improved: "Bacteria and fungi have various chemical composition, which strongly contributes to their turnover rates in soil: for bacteria it consists 2.3-33 days, whereas for fungi it accounts for 130-150 days (Moore et al., 2005; Rousk and Baath, 2007; Waring et al., 2013). Despite turnover of microorganisms directly effect the C turnover rates in intercellular compounds (cell membrane and cell wall biomarkers), this relationship has rarely been investigated so far. However, the comparison of C turnover for cell membrane and cell wall components can be used to characterize the contribution of various microbial groups to medium-term C utilisation and to the stabilization of microbially derived C in SOM".

5. I don't understand why there was not an attempt to estimate ks for the PLFAs. That would be the main purpose of this approach, in my view. Also, how can the VAM be building up 13C, if they are mycorrizhae? This probably suggest the marker was indicating a saprotroph, not mycorrhizae, which is known to happen in soils.

The decomposition constants can be estimated in our study, however, only for the groups where ^{13}C enrichment decreased. However, we fear that these values can not be obtained for all groups and thus, we can not make quality comparison. Yes, we agree that 16:1w5 can belong to saprotrophic fungi. This was corrected in the text.

6. About the aminosugars, what we still don't know and doesn't get explored and discussed, yet, it would be the most interesting is: which builds up more per unit of C assimilated (this would be an indication of which may contribute more to SOC building), or in other words a measure of enrichment/recovery.

Thank you for the comment. We can not estimate how much amino sugars were build per unit of C assimilated, because it is not possible to separate amino sugars of living microorganisms from one accumulated in SOM. However, we estimated enrichment of amino sugars by ^{13}C on the day 3: " To estimate the ^{13}C enrichment 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 is presented in the discussion section.

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7. Glucose is likely to behave differently to other C substrates, therefore I would restrict the title and Discussion to glucose (not carbon).

Title was corrected: Glucose C turnover in cell compartments and microbial groups in soil. The discussion was corrected and only glucose C was discussed.

8. The rationale for the difference found in turnover time between the cytosol and PLFA is really not convincing and not well supported. Both active and dormant organisms have a membrane and if they're not active they wouldn't be picking up the 13C.

Thank you for the comment. The idea of work was to estimate C turnover times in various cell microbial pools: cytosol, PLFAs and amino sugars. Cytosol is always assume as the most labile pool and supposed that C here should have the fastest turnover. However, according to results of our study, C turnover time in cytosol are much slower that in the structural cell pools (such as PLFAs).
Our data on ^{13}C pool enrichment includes information about both dormant and active microorganisms (due to enrichment was calculated relatively to the total cytosol C pool), and clearly shows, that more ^{13}C was incorporated into cell membranes than in cytosol. It also shows that C turnover is slower in cytosol, than in PLFAs. Concerning dormancy: even a dormant microorganisms repair membranes and, thus, have a C turnover in them, whereas microorganisms can be survived without reparation of other cell pools (such as DNA).

9.The explanation for why more 13C was in the bacterial than non-bacterial lipids completely ignores anything about their ecology or physiology.

Thank you for the comment. The goal of the study was to estimate the turnover times of C in various cell pools for native microbial community, which was already formed in the soil before we started the experiment. It was not a goal to investigate the ecology or physiology of microorganisms.
10.Describing what fatty acids were more or less abundant (section 3.3.) is not really informative as these data don't reflect absolute abundances and these patterns of abundance are more or less the same for a lot of soils.

Thank you for the comment. We have deleted this paragraph.

11. I would replace the term 'incorporation' with 'recovery' which seems to be what they calculated.

Thank you for the comment. The term 'incorporation' was change to 'recovery'.

12.I find it surprising that soil moisture would be "essentially constant" across 50 days.

The data for soil moisture were the following: 25.7±1.2 (3 days), 23.3±1.3 (10 days), 21.4±0.7 (50 days). This information was added into the material and method section. Due to we did not sample the soil directly after the rain, the moisture variations were low.

Minor methods comments The rational for the amount of C added is not presented.

The C was added in the amount to prevent any priming effects, as well as growth of microorganisms due to glucose application. The concentration was chosen to trace the natural

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pool of glucose in soil solution (Fischer et al., 2007), rather than stimulate the activity of microorganisms.

Not clear if the field collected soil column had or not vegetation.

The columns had no vegetation by the collecting time, as well as when the ^{13}C glucose was applied. This information was added into materials and methods section.

I don't understand the "assignment of fatty acids to distinct microbial groups by factor analysis".

Factor analysis was used as classification method, with the main purpose to split the microorganisms which belong to one group (according to literature) into subgroups which behave differently in the soil. Based on the analysis, fatty acids which were loaded into one factor with the same sign (+ or -) and were related to one bacterial or fungal group (based on literature), were summed together. In the end of the analysis only groups of PLFAs related to bacteria or fungi were presented. The relation of fatty acid to particular microbial group was taken from the literature (Zelles 1997). The information about assignment of fatty acids to distinct microbial groups was added into material and method section.

Reviewer 3.

Review of manuscript "Carbon turnover in cell compartments and microbial groups in soil" by Gunina et al.

The authors of this manuscript have analyzed the turnover of different cellular compounds/fractions for different microbial groups using a 13C labeling experiment (3, 10 and 50 days). This is clearly a worthy and important goal. The experiment is done well although the number of harvests (3) is minimal for this determination of turnover. For reasons described below, I think the manuscript is not acceptable for publication in its current form. The goal of the manuscript is to evaluate the turnover time of C in each pool and to assess the contribution of bacteria and fungi to SOM. A second goal is to determine the turnover time for different categories of microbes. They hypothesize that turnover time is short for cytosol, intermediate for PLFAs, and long for amino-sugars. However, the results they find indicate that turnover time of lipids<aminosugars<cytosol. They hypothesize that, based on aminosugar ratios, the bacteria contribute more to SOM than fungi, however, the results are contradictory (one ratio suggests bacteria, the other fungi). Instead of defending these observations and rejecting the hypotheses, complex reasons are proposed why turnover time of the cytosol is long, but it is still a "labile pool" that turns over fast but has tight cycling, and, in the discussion, it turns out that one of the aminosugar ratios is "better" than the other, so that the bacterial contribution to SOM is high. In other words, experimental results could not cause rejection of the hypotheses, therefore I have to conclude the experiment was poorly designed and not able to test the proposed hypotheses.

Thank you for evaluation of our study. We have defended our observations, concerning cytosol, because numerous previous data have reported much shorter turnover times of C in this pool. Moreover, cytosol is assumed as a labile pool, and we needed to explain why our

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initial hypothesis was rejected. Concerning amino sugars: application of ether galactosamine/muramic acids ratio or glucosamine/ muramic acids is a still a topic of discussion in soil science community. However, according to reviewer recommendations we excluded this from the discussion.

There are several reasons for the inability of this experiment to deliver results that are strong enough to test the hypotheses 1) It is unclear what "cytosol" is and why it is thought to be labile (L37). Although aminosugars and PLFAs are (bio)chemically distinct, this is less so for the fraction "cytosol" (L121, L179 and following). In order to understand the differences between lipids, aminosugars and "cytosol", the authors will have to analyze the amount of lipids and aminosugars in the cytosol fraction.

Thank you for the comment. Term "cytosol" was used by us for shortness. This pool is extracted by 0,05M K2SO4 after the chloroform fumigation. After measurement the C content in extracts from fumigated soil, we have substracted the amount of C extracted from the soil by 0,05M K2SO4 without fumigation. The reasons why cytosol is assumed as a labile pool due to : i) it contains significant amounts of carbohydrates (20-30%) (Joergensen et al., 1996), ii) it is a pool where the main chemical processes of the cell occur. Moreover, numerous researches have obtained much shorter turnover times of C in, than was obtained in our study. It is not possible to measure lipids in the cytosol pool, because lipids are not dissolved in 0,05M K2SO4 which is used to extract cytosol pool. Pool of amino sugars in cytosol fraction is several orders of magnitude lower than in acid hydrolysis fraction.

2) The experiment was not long enough to calculate turnover time for aminosugars (Fig. 2;). Moreover, although turnover is calculated using one exponential declining function (Fig. 2), in
the discussion, a whole paragraph is dedicated stating that glucose decomposition is bi-phasic (L
362), and so the use of a single exponential function needs to be defended.

Thank you for the comment. With the time scale which we have investigated, we were mainly focused on the second phase of glucose utilization. So, that is why the calculated half -life times of C are related to the second phase, and that is why we have used the single exponential model. Moreover, 3 time points do not enough for the double exponential model use. We have shorten the discussion about bi-phasic glucose decomposition.

Furthermore, conclusions about turnover rates are presented for PFLAs and aminosugars, without numbers to back up the conclusions. This is because of increasing 13C contents with time for aminosugars and fungal PLFAs; however, if the turnover times cannot be calculated, the conclusion should not be drawn, data should not have been presented (under this title) and/ or more data should be collected.

Thank you for the comment. Taken into account that,the ^{13}C replacement in amino sugars and fungal PLFA increased, it can partly prove that the C turnover times in these pools are longer, compare to the pools, where ^{13}C replacement started to decrease within the 50 days of experiment. We clearly wrote the problem about calculating the C turnover time for amino sugars in the discussion section, and did not do any specific conclusions about the data which we did not calculate.

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Additionally, turnover rates should have been calculated for the various bacterial and fungal groups based on PFLA data (according to the title). Finally, the presented turnover rates are presented without an estimate of the error associated with it (for example R2 value in Fig. 2, 3 and 4, SE for the turnover time values), making it impossible to evaluate whether the estimated turnover times for lipids and cytosol are truly different.

Thank you for the comment. Due to some PLFAs showed increase in ^{13}C replacement with time, we can calculate the C turnover rates only for the some PLFAs (which showed decrease in ^{13}C replacement). However, in that case we can not compare C turnover times for all microbial groups and can not make correct conclusions. However, if reviewer insists, we will make calculations for the groups, which showed decrease.

3) Hypothesis 1 is interesting, but cannot be tested in this experiment, as the initial uptake and incorporation in cytosol and other pools is fast. For example, Frey et al 2013 show that glucose uptake and incorporation in microbial "cytosol" occurs within 6 hours. The authors need to explain why and how this hypothesis can be tested using the experiment they designed. Hypothesis 3 is not a hypothesis but a (simplifying) assumption, used to interpret the results of this study, not a testable hypothesis. Moreover, the assumption is by definition wrong, but at best is an acceptable approximation. However, no evidence is given to support this assumption.
Is 50 days incubation still short enough that no aminosugars are transferred to the necromass pool? In general, the hypotheses are poorly defended or explained mechanistically.

Thank you for the comment. We agree with reviewer and removed hypothesis 3. We have improved hypothesis 1 and focus only on the turnover times: we hypothesised that 1) turnover times of C in pools follow the order cytosol<PLFA<amino sugars, because substances taken up by cells first are transported by membrane proteins into cytosol, from where they get distributed to other cellular pools. Moreover we have investigated the second phase of glucose C utilization, and not initial uptake, which was studied already many times.

Additional general comments
The statistics need to be further developed. The estimates of the turnover for the different fractions/compounds (L 304) need to be described with a mean and error. R2, significance and SE need to be added with Fig 2, 3 and 4. Current description does not make it possible to verify the assertion of the authors that the turnover rates of the various pools are significantly different. Fig. 5 does not add to understanding or interpretation of the results and can be removed.

Thank you for the comment. Significance and SE are presented in the figures 2, 3 and 4. Figure 5 is a synthesis of the ms results, however, if reviewer insists we will remove it.

The observation that the 13C incorporation (as a percentage) was higher in PLFA than in cytosol does not logically result in a conclusion that the incorporation is faster (L32). This result may just be a reflection of the size of the pool (PFLA versus "cytosol"), and certainly

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does not show “the importance” of membranes “for initial C utilization”.

Thank you for the comment. On the L32 we speak about enrichment of pool by ^{13}C - means % of ^{13}C in the total pool C, and this completely account for the pool size. The incorporation of ^{13}C does not account for the pool size, but we do not speak in L32 about that.

The use of the term filamentous organisms should be avoided. The authors probably mean fungi. I like the intent of L46, however, the comparison of the dynamic behavior of the three pools remains poorly developed.

Thank you for the comment. The term filamentous organisms can not be avoided, because we speak about fungi and actinomycetes (which can not be named as fungi). The dynamic behavior of the three pools was improved.

Careless use of references: L 68: Malik et al have not reported on cytosol, nor on its supposed heterogeneity. It is not at all clear how location would affect the turnover time of membranes and cell walls (L70).

Thank you for the comment. Malik et al investigated pool extracted after chloroform fumigation, which is partly reflect the composition of a cytosol pool. He measured molecular mass distribution and found out many fractions with different molecular weights, which is an observation of fraction heterogeneity. The term location was corrected to the term "function".

Bremer and Kuikman (1993; L 73) are not experts in microbial physiology, and therefore not an authorative source to support the statement that labeled glucose appears first in the “cytosol”. In fact, they only looked at the cytosol (fumigation-extractable) so cannot comment on whether other compounds or fractions become labeled first or later.

Thank you for the comment. We provided other reference:
Gottschalk, G.: Bacterial Metabolism, Springer-Verlag New York, New York, 1979.

A reference is needed to support the assumption that “the cytosol is considered to be the most dynamic pool within microbial cells”. Furthermore, heterogeneity (L75-76) has never stopped any calculation of turnover times, as is evident in soil organic matter turnover studies. Important references are missing for example those by Malik et al 2015 where comparison between “cytosol” and PFLAs are made (and DNA/RNA).

Thank you for the comment. The reference Malik et al 2015 was added. L75-76 we mean, that due to many different compounds are in the composition of cytosol (with different molecular masses) the turnover time of C in this pool is a mean of turnover times of these components. We have corrected: Organic compounds that are taken up by microorganisms first enter the cytosol (Bremer and Kuikman, 1994), which has a high heterogeneity in composition.

L96 and following: This paragraph tries to distinguish between cellular turnover – I assume as a consequence of cell death is what is referred to here – and turnover of compounds within a
living cell. However, it is not that easy to make that distinction – how does one distinguish between lipids being recycled and reused, taken apart and made into for example amino-acids,

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while other amino acids are recycled into lipids, and what happens after cell death – uptake of lipids by other organisms intact incorporated, reused, recycled, taken apart and/or turned into CO2. Moreover, the observations of increasing 13C concentrations for fungi versus decreasing
ones in bacteria suggests some transfer of compounds, but remains unexplained in this manuscript.

Thank you for the comment. We corrected:
Bacteria and fungi have various chemical composition, which strongly contributes to their turnover rates in soil: for bacteria it consists 2.3-33 days, whereas for fungi it accounts for 130-150 days (Moore et al., 2005; Rousk and Baath, 2007; Waring et al., 2013). Despite turnover of microorganisms directly effect the C turnover rates in intercellular compounds (cell membrane and cell wall biomarkers), this relationship has rarely been investigated so far. However, the comparison of C turnover for cell membrane and cell wall components can be used to characterize the contribution of various microbial groups to medium-term C utilisation and to the stabilization of microbially derived C in SOM.

L 146: it is not clear to me why unlabeled glucose was added to the control treatments.

Thank you for the comment. Unlabelled glucose was added to make the experimental conditions equal (C content of the additions). If we add only water, than conditions of the experiment would not be the same and we would not have true controls.

L 149: explain why the shelter was put in place and why it was removed. What was the effect of this on the soil moisture content?

Thank you for the comment. Shelter were putted to avoid the rain and the flow of glucose out the soil columns. The soil moisture remained nearly constant. The shelter were removed because this was field experiment and natural conditions should present on it.

L 157: why was the soil stored at 5 °C for 5 days prior to chloroform-fumigation analysis? What happened to the "cytosol" during that time? Does this mean that the value for cytosol is really the value after 8, 15, and 55 days?

Thank you for the comment. This is standard storage temperature for the soil sample before the analysis, if they can not be performed in the same day as sampling. Due to temperature in the field was around 15ºC, the storage at 5ºC will not cause strong effect on the cytosol fraction.

L 180: defumigation is not a word.

Thank you for the comment. Was changed to: "After removing the rest of chloroform from the soil....".

L 186: "extraction efficiency" not "extraction factor"

Thank you for the comment. Was changed.

L 247: "the assignment of fatty acids to microbial groups …" is confusing me. Does this mean

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that as part of this study, biomarker PFLAs are assigned to group independent of what is done in
other studies?

Thank you for the question. No, it means that we used previous studies to assign PLFAs measured in our experiment to the particular microbial groups. The appropriate reference is provided (Zelles, 1997).

L 249: this procedure is not clear to me, but I am not at all familiar with PFLA/Microbial community analysis. My first impression was that the analysis is basically a community analysis – showing, based on PFLAs, what the community looks like. However, L 247 suggests that with this procedure, PFLA are assigned to microbial taxa, but then in the heading of Supplementary Table 1 it suggests that literature data is used – Please clarify what the table is used for, how (and
what) literature data is used, and what the results of this analysis means for your experiment. Similarly, L 431: the arguments for using the 16:1w5 as a biomarker for VAM and not G- are weak. The abundance of VAM needs to be expressed relative to G- bacteria. Table one suggest
that the total C for PFLAs is higher than for VAM, thus is more abundant (?).

Thank you for the comment. The procedure is the following: PLFA analysis provides content of various PLFAs, some of them are specific for the groups of G+,G- bacteria, fungi, actinomycetes and arbuscular mycorrhizal fungi. This specifity based on the analysis of pure cultures (Zelles, 1997). Factor analysis with the principal component extraction method of mass % of individual PLFAs was done. The final assignment of fatty acids to distinct microbial groups was made by combination the results of factor loadings table with databases about presence of particular fatty acids in microbial groups (Zelles, 1997). Fatty acids which were loaded into the same factor with the same sign (+ or -) and belonged to one group (base of the table provided in Zelles (1997)) were related to one specific microbial group and their PLFA contents were summed. This method enables quality separation of microbial groups within the soils (Apostel et al., 2013; Gunina et al., 2014).
With PLFA analysis is not possible to conclude about the abundance of VAM and G- bacteria. Only the approximate calculated coefficient was proposed in literature (Baath and Anderson, 2003) for fungal biomass. That is why we spoke only about the content of biomarkers in our study, and not about biomass of microorganisms. Based on the PLFA-C it is not possible to conclude about the biomass of microbial groups.

L290: the description of the results (declines between 3 and 10 days but then remains constant
then constant) does not match the assumed exponential decline. Please explain.

Thank you for the comment. The information provided at the L290 is about recovery of the tracer in various pools, whereas exponential decline is seen for the enrichment of C in the pools (portion of ^{13}C in the total C pool).

Fig. 2, 3, 4: the statistical tests should also be done between harvests, not only between microbial groups.

Thank you for the comment. The statistical tests were added, where the differences between sampling points are clearly seen.

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L 347: the explanation for the differences between this study and published results, namely the
amount of glucose added and the microbial activity, are not revealed. Some further information
on these explanatory variables would be appreciated. Is microbial activity measured in this study, microbial activity is not measured? The idea that microbes store glucose when added in
small quantities is unproven – it is a mere assumption, recently defended by Sinsabaugh et al 2013, but evidence for storage was absent in recent experiments by Dijkstra et al (2015). The idea that the storage leads to maintenance is in contradiction to the 80% recovery after 50 days,
and with the idea that microbial pools and cells turn over fast.

Thank you for the comment. We have removed this paragraph from the ms.

L 362: the description of the two stages of glucose decomposition – 1) CO2 production plus biosynthesis, and 2) C incorporated in microbial cells is used for anabolism is confusing. Is anabolism different from biosynthesis? Is during the second phase CO2 production absent? How
do the two phases relate to the biosynthesis of lipids, cytosol, and aminosugars? Please clarify

Thank you for the comment. We used only the term biosynthesis. We wanted to highlight that during the first phase of glucose utilization the C is mainly goes to CO2, whereas during the second phase more C goes for biosynthesis, which includes reuse of cell compounds. In our study we focused on the second phase of glucose utilization.

L 395: what is this model, please explain some salient details and how it agrees with your observations.

Thank you for the comment. We have removed these sentences.

L 419: this rationalization needs some references or evidence that contact with the environment
leads to rapid turnover.

Thank you for the comment. Typical example for that is fast response of cell membranes to stress conditions (water stress, pH stress, pollution, lack of available C): formation of cyclo-fatty acids in membranes of gram-negative bacteria (Bossio et al., 1998, Guckert et al., 1986, Kieft et al.,1997).

L 421: the problem of active and inactive cells for cytosol dynamics is similar for lipid dynamics, as inactive cells also have membranes.

Thank you for the question. Actually the lipid behaviour is different from the cytosol pool: even inactive cells repair membranes, whereas other cell pool not necessary to be repaired. The typical example for that is: dormant microorganisms live with damaged DNA, but never with damaged membranes.

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L 482: how is this conclusion drawn when the turnover rate cannot be calculated according to L486. L 506: how do you determine that the turnover of the amino-sugars is higher than that of
the cytosol pool? L 509: this would be a wonderful conclusion, but it does not appear in the abstract at all. What is the reason that the cytosol is so stable? Please elaborate.

Thank you for the comment. Due to C replacement in amino sugars pool was still increasing after 50 days, we can not calculate the C turnover times. However, if replacement was still increasing, it is obviously that C turnover in amino sugars is slower than in PLFA and cytosol (where ^{13}C replacement decreased with time). The conclusion about: " 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" was included into the Abstract. The reason that cytosol is stable, is that it contains compounds with different molecular size (Malik et al., 2013): low molecular weight compounds, which renew fast and high molecular weight compounds which renew slow.

L 511 and following – the results from the measurements seem to indicate contrasting conclusions – bacteria or fungi are most important (L516 and following). It is then stated that only the galactosamine/muramic acid ratio should be used. So, this means that the reader has wasted a number of valuable brain cells thinking about the galactosamine/glucosamine ratios, and looked at the data, but that was all a waste of time? Why not start with what is known (galac/muramic ratio) and leave it at that. Furthermore, there is a lot more text about the three aminosugars and their ratios in relation to bacteria and fungi – is that still relevant inlight of L 521?

Thank you for the comment. We agree with reviewer, this part of discussion was reduced, and only galactosamin/muramic ratio was presented.

Fig 1: explain what is total 13C remaining, what is non-specified pool? Remake the Fig so that
the SE of the aminosugars are fully shown.

Thank you for the comment. Total 13C remaining is an amount of ^{13}C measured in bulk soil, it is a sum of ^{13}C in cytosol, PLFAs, amino sugars and non-specified pool. Non-specified pool is amount of ^{13}C in the soil without ^{13}C incorporated into cytosol pool, PLFAs and amino sugars. This explanation was added into the figure legend.

Fig. 2: what is the equation with the word “replacement” in it? I think it is just the function of 13C over time, and thus the word replacement can be removed, but I may be wrong. Add R2, P value and significance (and SE of the turnover estimate)

Thank you for the comment. This is enrichment, this is misprinting. This was corrected in the paper.

Fig. 3: instead of showing differences between microbial groups, we need to know the differences between dates AND microbial groups to evaluate how these differences represent significant differences in turnover, and whether this turnover differs between groups. Moreover, the goal of this paper was to determine differences in turnover between microbial groups, but this is not calculated. If turnover cannot be calculated for groups where 13C

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enrichment is increasing over time, what was the basis for the conclusion that turnover differed
between fungi and bacteria (L320)?

Thank you for the comment. The differences between dates were provided. Turnover of the different groups can be calculated only for one, which have decrease in ^{13}C replacement. Conclusion about the differences in turnover time of C between fungi and bacteria is made based on the trend of ^{13}C replacement: if ^{13}C replacement was still increasing it means, that the C turnover in particular PLFAs was longer compare to one where ^{13}C replacement was decreasing within the experimental time.

Fig 5: not really helpful.

Thank you for the comment. Fig. 5 was strongly improved: fluxes were clearly marked by the different size of arrows, position of x and y axises were changed. However, if reviewer insist we will remove it.

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Glucose C turnover in cell compartments and microbial groups in soil

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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% of total pool, resulting that the renewal of C in 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 cytosol is a pool with fast process rates, intensive recycling of cytosol components, within the living cells, leads to a longer C turnover time. Amino sugars originate mainly from microbial residues, thus longer experimental periods are required for estimation of C turnover times in that pool.

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. The results also reflect 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. This

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information is especially important for assessing C fluxes in soil and the contribution of C from microbial residues to soil organic matter.

Keywords

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

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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 (Malik et al., 2015) 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 functions, 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 (Gottschalk, 1979), which has a high heterogeneity in composition (includes components of various chemical structure and molecular weight). However, due to the heterogeneity of this pool, the calculated C turnover time is a mean of turnover times of various components. 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

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Удалено: 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.

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PLFA C in the living biomass are currently published. The formation of 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).

Bacteria and fungi have various chemical composition, which strongly contributes to their turnover rates in soil: for bacteria it consists 2.3-33 days, whereas for fungi it accounts for 130-150 days (Moore et al., 2005; Rousk and Baath, 2007; Waring et al., 2013). Despite turnover of microorganisms directly effect the C turnover rates in intercellular compounds (cell membrane and cell wall biomarkers), this relationship has rarely been investigated so far. However, the comparison of C turnover for cell membrane and cell wall components can be used to characterize the contribution of various microbial groups to medium-term C utilisation and to the stabilization of microbially derived C in SOM.

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Удалено: 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)

Удалено: 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 they can be stabilized in SOM as polymers (Glaser et al., 2004). Thus, the

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

We analyzed glucose derived ^{13}C partitioning into the cytosol, cell membranes and cell walls, to evaluate the turnover time of C in each pool, and to assess the contribution of bacterial and fungal biomass to SOM. We hypothesized that: 1) turnover times of C in pools follow the order cytosol<PLFA<amino sugars, because substances taken up by cells first are transported by membrane proteins into cytosol, from where they get distributed to other cellular pools and 2) incorporation of ^{13}C glucose should be faster and higher for bacterial than for fungal biomarkers, because bacterial biomass has a faster cell turnover than fungal biomass.

Удалено: We hypothesized that: 1) C from sugars is first incorporated into the cytosol, and subsequently into structural compartments such as cell membranes (PLFA) and cell walls (amino sugars). Thus, the turnover times should increase in the order:

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Удалено: ; 3) due to amino sugars have long turnover times and are mainly dominated in microbial necromass, all incorporated ^{13}C can be related only to living biomass and allow estimate percent of replaced C in amino sugars of living microorganisms.

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2. Material and Methods

2.1. Field site and experimental design

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

In summer 2010, following harvest of the triticale, columns (diameter 10 cm and height 13 cm) were installed to a depth of 10 cm. Each column contained 1.5 kg of soil and bulk density was 1.36 g cm^{-3}. The 50 mL of uniformly labelled ^{13}C glucose (99 atom % 13C) was injected into the columns via a syringe at five points inside the column to spread the tracer homogeneously. Syringe was equipped with a special pipe having length 13 cm and perforated along the whole length, while the end of the pipe was sealed to prevent glucose injection below of the column. Each column received 93.4 µmol ^{13}C of tracer (0.06 µmol ^{13}C g^{-1}soil) and similar amounts of non-labeled glucose were applied to the control columns, to make the experimental conditions equal. The concentration was chosen to trace the natural pool of glucose in soil solution (Fischer et al., 2007), rather than stimulate the activity or growth of microorganisms.

The experiment was done in four field replicates, which were organized in a randomized block design. Labelled and control columns were present within each block. For the first 10 days of the experiment the rainfall was excluded by protective shelter, which was then removed and the experiment was run for 50 days in total. The rainfall was excluded to prevent the added glucose to be leached out from the soil profile, due to processes of microbial uptake go slower in the field conditions, than in the controlled laboratory. After 3, 22

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10 and 50 days, separate soil columns (four columns where ^{13}C was applied and four control columns) were destructively sampled. The columns had no vegetation by the collecting time, as well as when the ^{13}C glucose was applied.

The soil was removed from the column, weighed and the water content was determined in a subsample. Soil moisture was determined by drying samples for 24 h at 105 °C and was essentially constant during the experiment, ranging between 21–25% (25.7±1.2 (3 days), 23.3±1.3 (10 days), 21.4±0.7 (50 days)). Each soil sample was sieved to <2 mm and divided into three parts. One part was stored frozen (-20ºC) for PLFA analysis, another was cooled (+5°C) (during one week) before the microbial biomass analysis, and the rest was freeze-dried and used for amino-sugar analysis and for measurement of the total amount of glucose derived ^{13}C remaining in the soil.

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

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

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

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

with:

$[C]_{soil/BG/glc}$ C amount of enriched soil sample / background soil sample /

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	glucose derived C in soil	(mol · g_{soil}^{-1})
at%$_{soil/BG/glc}$	^{13}C in enriched soil sample / background soil sample / applied glucose	(at%)

2.3. Cytosolic C pool

The cytosolic pool was determined by the fumigation–extraction technique from fresh soil shortly after sampling, according to Wu et al. (1990) with slight changes. Briefly, 15 g fresh soil was placed into glass vials, which were exposed to chloroform during 5 days. After removing the rest of chloroform from the soil, the cytosolic C was extracted from the soil with 45 mL 0.05 M K_2SO_4. As fumigation–extraction technique allows to obtain not only soluble components, but also cell organelles and cell particles, we named pool of C in fumigated extracts as cytosol only for simplification of terminology. Organic C was measured with a high-temperature combustion TOC-analyser (Analyser multi N/C 2100, Analytik Jena, Germany). The cytosolic pool was calculated as the difference between organic C in fumigated and unfumigated samples without correcting for extraction efficiency. After organic C concentrations were measured, the K_2SO_4 extracts were freeze-dried and the δ^{13}C values of a 30–35 µg subsample were determined using EA-IRMS (instrumentation identical to soil δ^{13}C determination). The recovery of glucose derived ^{13}C in fumigated and unfumigated samples was calculated according to the above-mentioned mixing model (Eq. 1 and 2). The ^{13}C in the microbial cytosol was calculated from the difference in these recoveries.

2.4. Phospholipid fatty acid analysis

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

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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 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 Eq. 1 and 2, following a two-pool dilution model (Gearing et al., 1991).

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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,

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galactosamine, mannosamine and muramic acid) as external standards at four different concentrations (Dippold et al., 2014).

2.6. Calculations and statistical analysis

Factor analysis with the principal component extraction method of mass % of individual PLFAs was done. The final assignment of fatty acids to distinct microbial groups was made by combination the results of factor loadings table with databases about presence of particular fatty acids in microbial groups (Zelles, 1997). Fatty acids which were loaded into the same factor with the same sign (+ or -) and belonged to one group (base of the table provided in Zelles (1997)) were related to one specific microbial group and their PLFA contents were summed. 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.

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

Удалено: The assignment of fatty acids to distinct microbial groups was performed by factor

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$^{13}C_{Applied}$	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	(%)
k	decomposition rate constant	(% day^{-1})
t	time	(days)

One-way ANOVA was used to estimate the significance of differences in total ^{13}C recovery 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).

Удалено: incorporation

3. Results

3.1. Glucose utilisation and its partitioning within microbial biomass pools

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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 highest recovery of ^{13}C was found for cytosol pool (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 recovery 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 ^{13}C recovery into PLFA was generally very low and was in the same range as recovery into amino sugars (Fig. 1). The ^{13}C recovery in PLFA showed no clear trend between the sampling points (high standard error) (Fig. 1). In contrast, ^{13}C recovery in amino sugars increased two fold on the 50th day experiment ($p<0.05$).

[Fig. 1]

3.2. Turnover time of C in microbial biomass pools

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

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Удалено: amount

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Удалено: was 5–8 times lower in

Удалено: cytosolic pool. The ^{13}C enrichment in

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[Fig. 2]

3.3 Phospholipid fatty acids

Fatty acids of bacterial origin dominated over those of fungal origin within the living microbial community characterized by PLFA composition (Table 1). The PLFA content of most groups did not change significantly during the experiment, reflecting steady-state conditions for the microbial community (see Suppl. Table 2).

Glucose derived ^{13}C was incorporated in higher portions into bacterial than into fungal PLFAs (Fig. 3, top). Remarkably, the ^{13}C enrichment decreased over time for all bacterial PLFAs, whereas it increased or remained constant for 16:1ω5, fungi and filamentous, bacterial actinomycetes (Fig. 3, bottom), indicating differences in C turnover in single-celled organisms compared to filamentous organisms.

[Fig. 3]

3.4. Amino sugars

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

The recovery of glucose derived ^{13}C into amino sugars increased in the order: muramic acid = galactosamine < glucosamine (Fig. 4, top) reflecting partly their pool sizes. The ^{13}C recovery showed no increase from day 3 to day 50 for any amino sugars. The ratios

Удалено: Gram-negative (G-) fatty acids were more abundant than gram-positive (G+) ones. Actinomycetes and vesicular arbuscular mycorrhiza (VAM) fatty acids dominated in the composition of filamentous microorganisms, and saprotrophic fungi showed a relatively low presence in PLFAs.

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of glucosamine/muramic acid and galactosamine/muramic acid, calculated for the incorporated ^{13}C, were about six. This is much lower than the ratio observed for the pools of amino sugars. The ^{13}C enrichment did not increase from day 3 to day 50 for any of the amino sugars. The highest enrichment was observed for muramic acid and the lowest for galactosamine (Fig. 4, bottom). The ^{13}C enrichment in amino sugars was 10–20 times lower than for PLFA.

[Fig. 4]

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 (Bremer and Kuikman, 1994; Schneckenberger et al., 2008), as well as differences in microbial activity (Bremer and Kuikman, 1994; Schimel and Weintraub, 2003) in the investigated soils, explain the variation between studies in the portion of remaining glucose C.

The highest mineralization of glucose derived ^{13}C (20 %) was found within the first three days after tracer application (Fig. 1), whereas at day 50 mineralization was much slower. 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 and second one, when C incorporated into MB is further transformed and is used for microbial biosynthesis and mineralization of glucose-C to CO_2

Отформатировано: Поз.табуляции: 15,24 см, по левому краю

Удалено: 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).

Отформатировано: Шрифт: не курсив

Удалено: decomposition of 20%

Удалено: (Fig.

Удалено: 1) agrees with previously reported data

Код поля изменен

Удалено: (Boddy et al., 2007; Gregorich et al., 1991; Perelo and Munch, 2005)

Удалено: ¶ ¶

occurs much slower (Bremer and Kuikman, 1994). 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). Due to the first sampling point in our experiment was 3 days after glucose addition, the obtained data on glucose mineralization can be mainly related to the second stage.

A significant portion of glucose derived C was stored in the non-specific pool in SOM (Fig. 1), e.g., as microbial storage compounds and other cellular building blocks, which can contribute to C accumulation in microbial residues (Wagner GH, 1968; Zelles et al., 1997; Lutzow et al., 2006). This part cannot be extracted by the methods applied in this study. The amino sugar method detects only the peptidoglycan and chitin proportions of the cell walls, whereas other constituents can not be determined by this method (Glaser et al., 2004). Chloroform fumigation only partially extracts the cytosolic cell compounds, as high molecular weight compounds, which interact with the soil matrix, cannot be extracted with low molarity salt solution.

4.2. Partitioning of ^{13}C-derived glucose between cell compounds

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

Удалено: . Glucose is decomposed in soil in two stages

Удалено: (Gunina and Kuzyakov, 2015)

Удалено: : during the first one, part of glucose C is immediately mineralized to CO_2 and part is incorporated into the microbial compartments

Код поля изменен

Удалено: , during which C incorporated into MB is further transformed and is used for microbial anabolism e.g., in stable cell polymers, or stored for later catabolism (Bremer and Kuikman, 1994).

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4.3. Cytosol

We calculated the ^{13}C enrichment of the cytosolic microbial C pool, extracted after chloroform fumigation. The estimated turnover time of C in this pool was about 151 days. 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). The long C turnover time in cytosol is related to the high heterogeneity of this pool, which includes compounds with various molecular masses (Malik et al., 2013) and functions, having different turnover times. Thus, C turnover time in cytosol presents the mean value of turnover times of these compounds.

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 recovery 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

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Удалено: 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.

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Отформатировано: Отступ: Первая строка: 0 см

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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 an approach that enables direct estimation of the turnover of freshly added C by the initial incorporation peak. The approach used in the present study showed that the C turnover time in PLFA is about 47 days (Fig. 2). Accordingly, if the decomposition after cell death is about three days, the PLFA turnover time in living cells is about 44 days. This short turnover time of PLFAs is significantly lower than the C turnover time in the cytosol (Fig. 2). This is because the membrane is the interacting surface between the cell and the environment and thus, frequent and rapid adaptations of its structure are crucial for active microorganisms (Bossio et al., 1998, Kieft et al.,1997). In contrast, the extracted cytosolic pool includes C from both active and dormant microorganisms (Blagodatskaya and Kuzyakov, 2013), and the latter can dilute the ^{13}C signal incorporated into the active pool with non-labelled C, yielding a lower turnover of this pool.

4.4.2. Contribution of microbial groups to glucose derived C utilisation

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

The ^{13}C recovery into gram-negative fatty acids was higher (taking both G- groups together) compared to G+ bacterial PLFAs (Fig. 3, top), which might be due to: i) the abundance of their fatty acids, which was higher (Table 1) or ii) glucose uptake activity, which was higher for G- than G+ groups. In contrast, the ^{13}C enrichment (^{13}C recovery

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

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related to total C in particular biomarkers) for G- bacterial PLFAs was not higher than that for G+ (Fig. 3, bottom). Thus, the high ^{13}C recovery into G- bacterial biomarkers can mainly corresponds to their high content in the soil, not to higher activity of microbial groups. However, replacement of PLFAs C by glucose derived ^{13}C is only a proxy of microbial activity and can only partly estimate the real activity of microbial groups. This clearly suggests that the analysis of isotope data after labeling in general requires the calculation and combined interpretation of both the total tracer C recovery as well as the ^{13}C enrichment in the investigated pool.

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

The ^{13}C enrichment of bacterial PLFAs decreased from day 3 to day 50, whereas ^{13}C in fungal PLFAs increased (in the case of 16:1ω5) or stayed constant (Fig. 3, bottom). The decrease in ^{13}C enrichment in bacterial fatty acids indicates a partial turnover of bacterial lipid membranes, which is much faster than turnover in fungal membranes. This result is consistent with the turnover time of bacterial biomass in soil (Baath, 1998), which is about 10 days, whereas fungal biomass turnover times range between 130–150 days (Rousk and Baath, 2007). Consequently, the increase in ^{13}C enrichment in fungal PLFAs at late sampling points indicates that fungi consume the exudation products of bacteria or even dead bacterial biomass (Zhang et al., 2013; Ziegler et al., 2005).

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4.5. Amino sugars

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

Amino sugars represented the largest microbial pool investigated in this study (Table 1) and comprised 3.7% of SOC. Chitin and peptidoglycan, the direct sources of the amino sugars, comprise no more than 5% of cell biomass (Park and Uehara, 2008; Wallander et al., 2013). Therefore, the high amount of amino sugars relative to PLFA can only be explained by their high proportion in microbial residues/necromass (Glaser et al., 2004; Liang et al., 2008; Glaser et al., 2004). Irrespective of the large pool size of the amino sugars, their recovery and pool enrichment with glucose derived ^{13}C was the lowest compared to other compartments in living cells and increased during the experiment. Consequently, amino sugars can have the slowest turnover in soils, presumably even within living cells, for two reasons: 1) cell walls are polymers that require a rather complex biosynthesis of the amino-sugar fibers, 2) cell-wall polymerization occurs extracellularly (Lengeler et al., 1999) and 3) microorganisms do not need to synthesize peptidoglycan unless they multiply. To calculate C turnover time in this pool, conducting of long-term experiments is 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 recovery 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

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Удалено: enrichment of amino sugars

Удалено: from day 3 to day 50, in contrast to cytosol and PLFA pools.

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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 recovery 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).

4.5.2. Contribution of bacterial and fungal cell walls to SOC

Glucosamine was the dominant amino sugar in the soil, whereas muramic acid was the least abundant (Table 1), which agrees with most literature data (Engelking et al., 2007; Glaser et al., 2004). To conclude about the proportions of bacterial and fungal residues in the SOM, the ratio of galactosamine/muramic acid (Glaser et al., 2004) was calculated (Table 1) and showed bacteria to be the dominant within the soil microbial community. The bacterial origin of microbial residues in the soil is supported by: 1) the dominance of bacterial PLFA biomarkers 2) the environmental conditions of the site, namely, long-term agricultural use, which promotes the dominance of bacterial communities.

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Удалено: , whereas the glucosamine/muramic acid ratio indicated that the fungal contribution was larger. This discrepancy is due to unknown sources of glucosamine in the soil (Glaser et al., 2004), i.e. it originates from bacterial (Amelung et al., 2001b) and fungal cell walls (Fernandez and Koide, 2012; Glaser et al., 2004; Park and Uehara, 2008) as well as from actinomycetes, insect and invertebrate. Moreover, previous studies have confirmed galactosamine to be the most characteristic amino sugar for fungal residues in soil (Engelking et al., 2007) and thus, galactosamine/muramic acid ratio is more useful for estimation the composition of microbial residues in SOM

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

The calculated ^{13}C enrichment was the highest in muramic acid (Fig. 4, bottom). This is in agreement with the high ^{13}C enrichment of bacterial PLFAs compared to 16:1ω5 and fungi (Fig. 3). Due to differences in cell-wall architecture, G+ bacteria contain more muramic acid (approximately four times) than G- bacteria (Lengeler et al., 1999), and thus make a higher contribution to the ^{13}C enrichment of muramic acid.

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

5. Conclusions

Tracing the ^{13}C labelled glucose through cytosol, PLFAs and amino sugars is a prerequisite for understanding the fate of organic substrates in soil. The highest ^{13}C enrichment, and thus turnover of C, was found for the PLFA pool, corresponding to a turnover time of 47 days,

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whereas the turnover was slower in the cytosol (150 days), which contradict to the first hypothesis. Such results can be explained by 1) efficient C recycling in the cytosol, and 2) its heterogeneous composition, which contains compounds with different turnover rates. The ^{13}C enrichment of amino sugars was still increasing at the end of the experiment, reflecting the slowest C turnover within the investigated pools and that most of this pool consisted of microbial residues and not living biomass. An approximate calculation of ^{13}C enrichment of amino sugars in the living biomass gave values 0.57% of pool size, which was still lower than for PLFAs. Thus, C turnover in membrane components is faster than in cell wall components, even if only the portion of the amino sugar pool in living biomass is considered.

Bacterial PLFAs dominated in the microbial community composition, and much higher glucose derived ^{13}C was incorporated to bacterial than to fungal PLFAs too. This agrees with prevailing role of bacteria in the utilisation of easily available organic substrates that are present at low concentrations in soil. A lower ^{13}C enrichment of filamentous PLFAs compare to bacterial can evidence that i) C turnover in filamentous PLFAs is slower compare to bacterial and ii) filamentous organisms might utilize the products of bacterial metabolism and biomass.

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

Consequently, the combination of ^{13}C labeling with the subsequent analysis of several microbial cell compartments and biomarkers is a unique approach to understanding C partitioning within microbial cells and the microbial communities in soil. This knowledge is

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Удалено: A slower turnover of filamentous and mainly fungal biomass compared to bacteria was observed in the ^{13}C enrichment of the respective PLFAs. Therefore,

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not only crucial for assessing C fluxes and recycling in soil, but is also of special importance concerning the contribution of C from microbial residues to SOM.

Author contribution

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

Data availability

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

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Table 1 Amount of microbial biomass compartments, their C content, PLFA content of microbial groups and composition of microbial residues in investigated soil. G-1 and G-2 are gram-negative group one and two, respectively; G+1 and G+2 are gram positive group one and two, respectively; Ac – actinomycetes; 16:1ω5 - saprotrophic fungi. Data present mean of three time points (with four replications for each time point) ± 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	
16:1ω5	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

*Data are taken from Glaser et al. (2004).

Удалено: VAM - vesicular arbuscular mycorrhiza

Отформатировано: не выделение цветом

Удалено: VAM

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Table captions

Table 1 Amount of microbial biomass compartments, their C content, content of microbial groups and composition of microbial residues in investigated soil. G-1 and G-2 are gram-negative group one and two, respectively; G+1 and G+2 are gram positive group one and two, respectively; Ac – actinomycetes; 16:1ω5 - saprotrophic fungi.

Удалено: VAM - vesicular arbuscular mycorrhiza

Figure captions

Fig. 01 Partitioning of glucose derived ^{13}C in SOM presented as the ^{13}C recovery (% of initially applied ^{13}C found in soil) between the following pools: non-specified SOM (calculated as total ^{13}C recovery subtract ^{13}C recovery in cytosol, PLFAs and amino sugars), cytosolic, PLFAs and amino sugars. Small letters reflect differences between the sampling points for the distinct pool. Data present mean (n=4) and bars present standard errors (SE). The SE for the amino sugars are not fully shown.

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

Fig. 03 Recovery of glucose derived ^{13}C (top) and ^{13}C enrichment (bottom) of the microbial PLFAs. Note that the values for 16:1ω5 and fungi are scaled-up 10 times (secondary Y axis) compared to those of other groups (Y axis at the left). Data present mean (n=4) and bars

Удалено: Total incorporation

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present standard errors. Small letters reflect differences between the microbial groups for ^{13}C recovery and ^{13}C enrichment from glucose; letters a-d are for day three, l-o are for day 10, x-z are for day 50.

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

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

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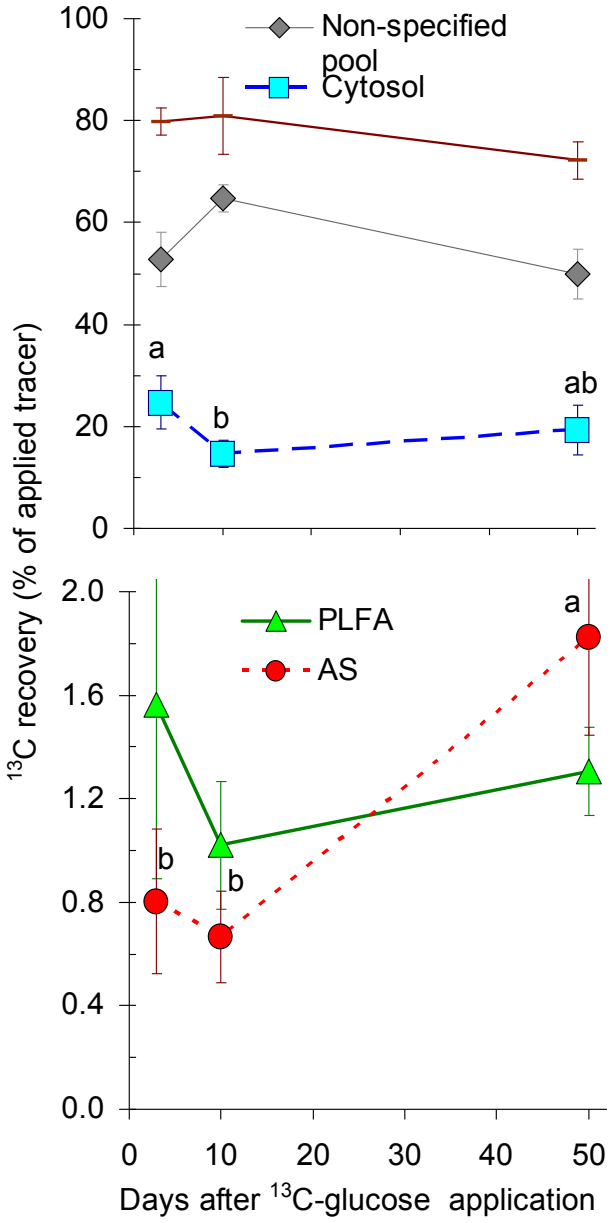
Figure 01.



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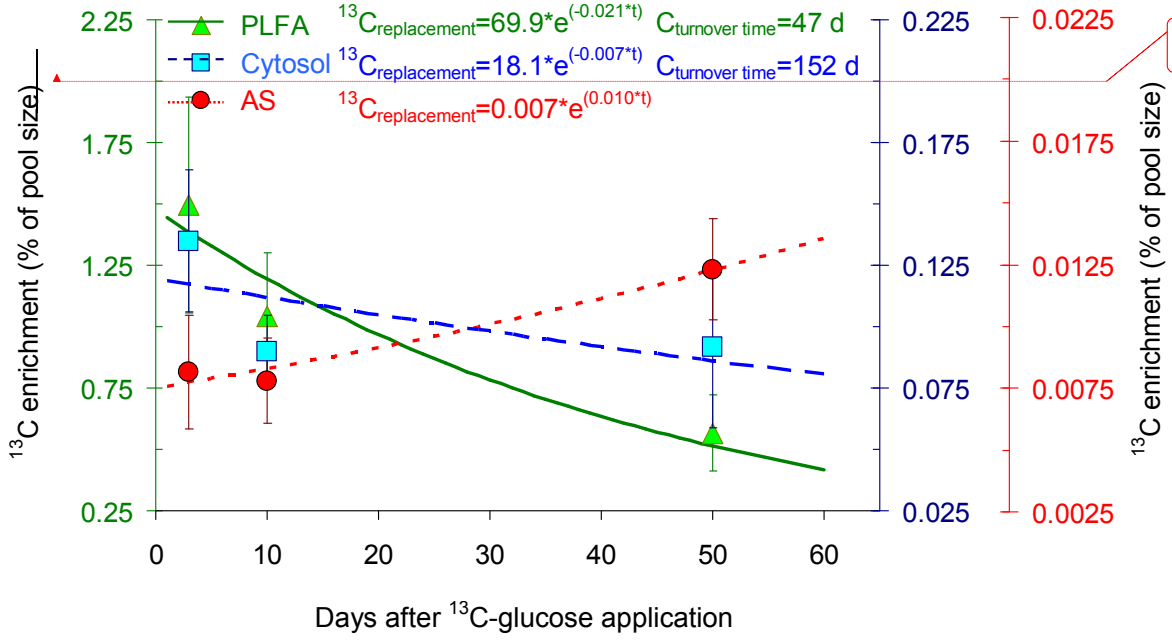

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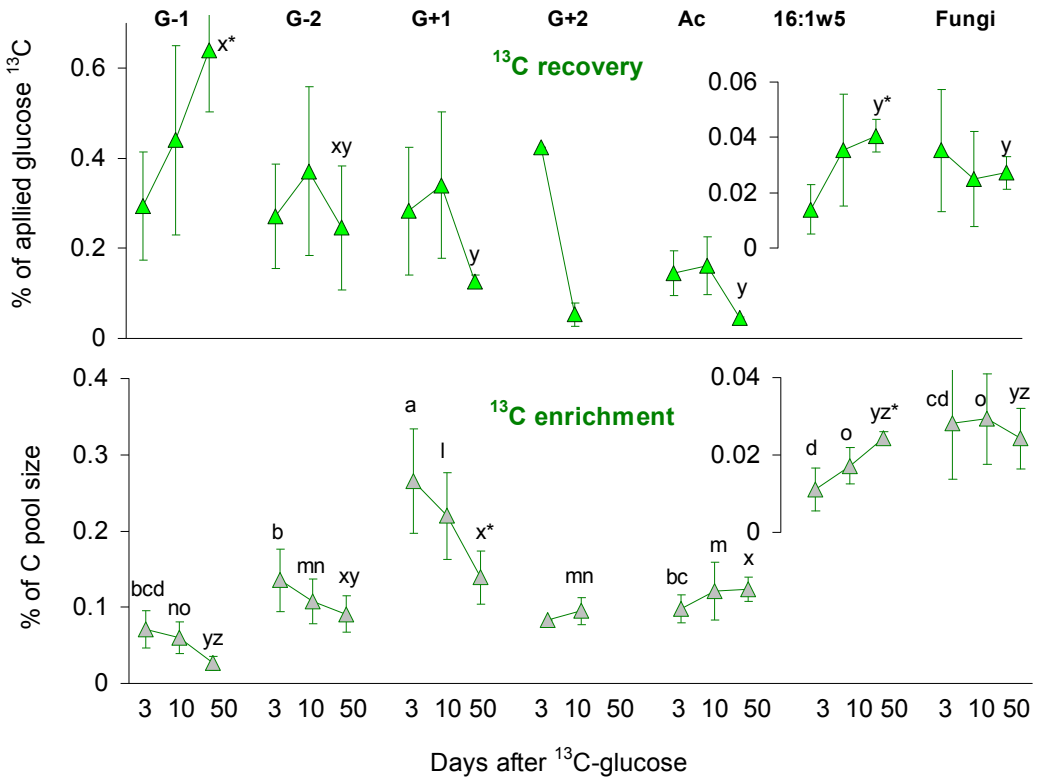
Figure 03.



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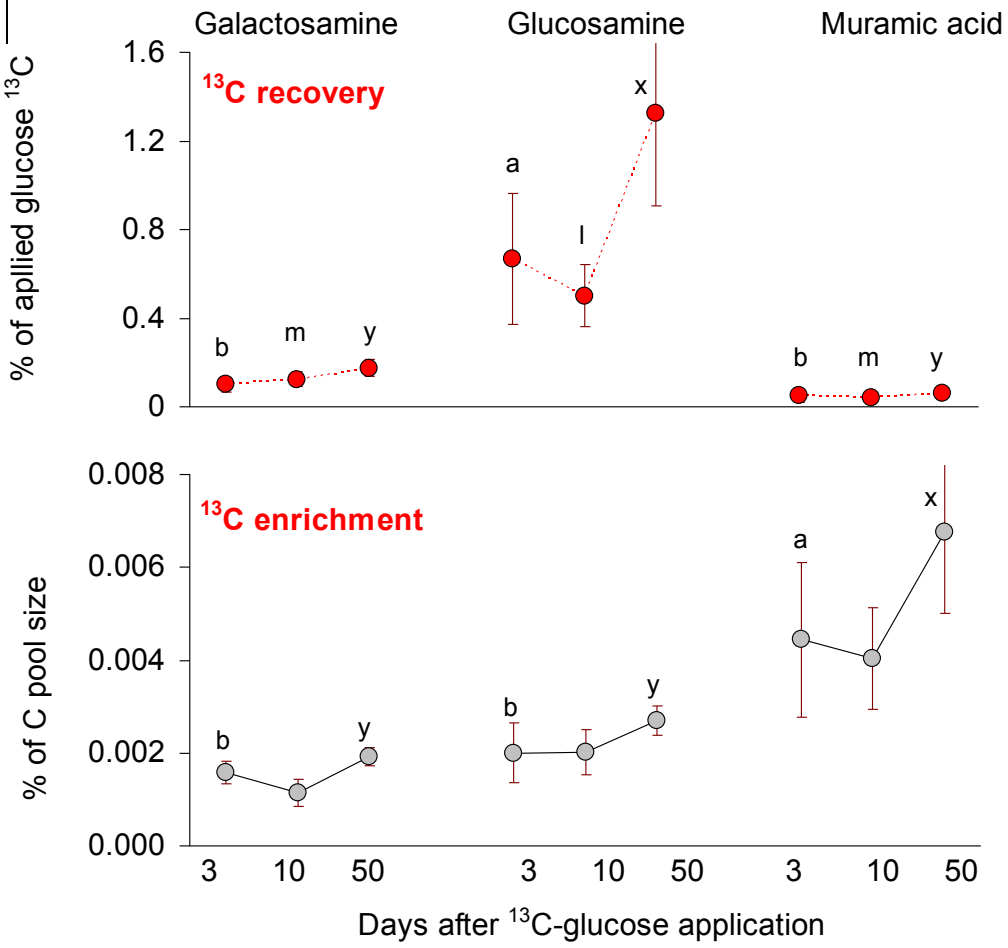
Отформатировано: немецкий (Германия)

890 Figure 04.



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