Biogeosciences Discuss.,
doi:10.5194/bg-2016-214-AC3, 2016


Biogeosciences Discussions

# *Interactive comment on* "Carbon turnover in cell compartments and microbial groups in soil" *by* Anna Gunina et al.

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Received and published: 20 August 2016

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. Moreover, cytosol is assumed as a labile pool, and we needed to explain why our 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 the soil science community, however, according to reviewer recommendations we will exclude this from the discussion and will shorten this section significantly.

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 is pool extracted by 0,05M K2SO4 after the chloroform fumigation and substraction 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 one extracted by acid hydrolysis.

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.

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. If the 13C replacement in amino sugars and fungal PLFA increase, it is obviously that the C turnover is longer, compare to the pools, where 13C replacement started already decrease within the 50 days of experiment. However, we clearly wrote this problem in the discussion section and did not to any specific conclusion about the data which we did not calculate. The title of the ms can be changed to: "Glucose C turnover in microbial cell compartments in soil", to avoid confusion for the readers.

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 still increase in 13C replacement, we can calculate the C turnover rates only for the some PLFAs (which showed decrease in 13C replacement). However, in that case we can can not compare C turnover times for all groups and make a correct conclusions. However, if reviewer insist, we will make calculations for the groups, which showed decrease. The R2 values will be provided for the figure 2.

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 hypotheses that 1) turnover times of 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 in the figures 2, 3 and 4. R2 will be added to the figure 2. Figure 5 is a synthesis of the ms results, however, if reviewer insist 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 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 13C - means % of 13C in the total pool C, and this completely account for the pool size. The incorporation of 13C 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 will be 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 fraction with different molecular weights, which is an observation of fraction heterogeneity. The tern location will be 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 will provide other references: Bacterial Metabolism (G. Gottschalk, 1986).

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 will be added. L75-76 we mean, that due to many different compounds are in cytosol composition (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, 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 will deep this problem in introduction section, and will provide some possible explanations of 13C increase in fungal PLFA (cross feeding, recycling of 13C from microbial metabolites).

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 from 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 day. No, due to temperature in the field was around 15°C, the storage at 5°C 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 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. These specifity was established based on the analysis of pure cultures (Zelles, 1997). Based on the factor analysis table of factor loadings was obtained, and within one factor the fatty acids with the same sign (+ or -) and which are belong to one group (base of the table provided in Zelles, 1997) were related to one specific group and content of their PLFAs were summed up. 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 13C 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 will be added, where the differences between sampling points are clearly seen.

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. More information about the effect of amount of added glucose will be added. No, microbial activity was not measured. We agree with the reviewer and remove maintenance from the ms. We mean rather incorporation into cell



components than mineralization.

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 will use 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 were focus 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. In revised version we will remove 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 behavour is different from the cytosol pool: even a 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.

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 is still increasing after 50 days, we can not calculate the C turnover times. However, if replacement is still increasing it is obviously that C turnover in amino sugars is slower than in PLFA and cytosol. 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" will be included into abstract. The reason that cytosol is stable is that in contains compounds with different molecular size (Malik et al., 2013): as low molecular weight, which renew fast and high molecular weight 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 par of discussion will be reduced, and only galac/muramic ratio will be 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 13Đą remaining is a rest amount of 13C measured



in bulk soil, it is a sum of 13C in cytosol, PLFAs, amino sugars and non-specified pool. Non-specified pool is amount of 13C rest in the soil without 13C incorporated into cytosol pool, PLFAs and amino sugars. This explanation will be added into the material and method section.

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 will be 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 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 will be provided. Turnover of the different groups can be calculated only for one, which have decrease in 13C replacement. Conclusion about the differences in turnover time of C between fungi and bacteria is made based on the trend of 13C replacement: if 13C replacement is still increasing it means, that the C turnover in particular PLFAs is longer compare to one where 13C replacement 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.