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 ^{13}C 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.

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.
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. 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. 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.
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.

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

- 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?
- L 180: defumigation is not a word.
- L 186: "extraction efficiency" not "extraction factor"
- 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?
- 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 (?).
- 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.
- Fig. 2, 3, 4: the statistical tests should also be done between harvests, not only between microbial groups.
- 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.
- L 362: the description of the two stages of glucose decomposition – 1) CO_2 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 CO_2 production absent? How do the two phases relate to the biosynthesis of lipids, cytosol, and aminosugars? Please clarify
- L 395: what is this model, please explain some salient details and how it agrees with your observations.
- L 419: this rationalization needs some references or evidence that contact with the environment leads to rapid turnover.
- L 421: the problem of active and inactive cells for cytosol dynamics is similar for lipid dynamics, as inactive cells also have membranes.
- L 482: how is this conclusion drawn when the turnover rate cannot be calculated according to L 486. 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.

- 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?
- 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.
- Fig. 2: what is the equation with the word "replacement" in it? I think it is just the function of ^{13}C over time, and thus the word replacement can be removed, but I may be wrong. Add R^2, P value and significance (and SE of the turnover estimate)
- 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 ^{13}C enrichment is increasing over time, what was the basis for the conclusion that turnover differed between fungi and bacteria (L320)?
- Fig 5: not really helpful.

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