Review of BG-2016-317 by Paul et al.

This study investigates the hydrogen dynamics in soil organic matter to quantify processes such as the preservation of organic matter and microbial biosynthesis. Seems to be that this research is potentially useful to understand the fate of tritium (^{3}H) in ecosystems. The approach described in this paper for determining the fate of hydrogen in soil systems using three types of labelling experiments (substance, substance/water, only water) is an original approach. However, my major comment is related to the assumptions used for hydrogen exchangeability, which were poorly explained. I believe this manuscript needs significant explanation about the hydrogen isotope analyses and modelling. I therefore recommend publication only after major revisions.

H exchangeability – Soil organic matter could be a heterogeneous material in terms of hydrogen exchangeability. Uncontrolled isotopic exchange between sample and laboratory ambient vapour can introduce bias in δ^2H measurements. The authors did not explicitly account for H exchangeability in their analysis by using the Comparative Equilibration method or the aid of devices that allows vapour equilibration before analysis. Moreover, bulk soil samples without lipid extraction was conducted. As the authors pointed out, lipids do not usually exchange with atmospheric vapour because of the C-H bonds in their main structure. However, differential lipid content in bulk soil might bias the δ^2H measurements as well. In this study, non-exchangeable standards of non-similar matrix to the samples were run for calibration and hydrogen exchangeability seem to be corrected by measuring labeled and unlabeled samples at the same time. In theory, this could be a reasonable way to deal with this issue, but the authors should provide more details.

Specific comments

p4, line 106: Residual soil moisture is of great relevance when determining H isotope measurements because it would be a reservoir of H in the sample to be analyzed. Was it estimated once at the beginning of the experiment? Was performed after collection from incubators and freeze-drying? This step is crucial to eliminate any ‘contamination’ of residual moisture from the experiments.

p4, line 109: Please confirm amounts of water added.

p4, line 108: Provide uncertainty associated with this value.

p4, lines 134-140: One striking thing is the incubation experiment protocols. The authors opened the incubation systems every two days during the first three weeks and then every week. I understand this is important to keep aerobic conditions along the experiment. Would this compromise the ^{2}H abundance of the water? Further explanation is required here.

p5, line 147: For how long were samples freeze-dried? Again, this step is further relevant to eliminate possible contamination of ‘deuterated’ moisture in the sample to be analyzed. Previous investigations with organic materials have found that long periods of drying are needed.

p5, lines 153-162: Needs a more detailed description of the analyses. For example, a merit of precision using this method based on the standards measured is needed. How the ^{2}H abundance of water was measured?

More importantly, how the authors deal with the hydrogen exchangeability is quite reduced in the manuscript and relatively obscured to the reader. In the section 2.4, the authors only stated the following sentence: “Labeled and unlabeled samples were kept under the same atmosphere until the final $\delta^{2}H$ measurement.” Would that mean that they conducted a comparative equilibration method? This method is extensively used in the literature, but mostly for natural abundance samples. Any modifications for labeled samples are required? How long were the samples left under the same atmosphere? Which atmosphere? Laboratory atmosphere? Or inside a desiccator and then opened to the laboratory ambient? In short, the authors need to provide more details in their methodological section.

Another question I have is whether the use of two reference materials for calibration that cover a very small range of delta values (~2 per mil for $\delta^{13}C$ and ~20 for $\delta^{2}H$) can adversely affect the accuracy of their measurements of labeled samples among runs. Do the authors consider the use of a labelled standard?

Were other analytical issues such as memory effects considered? Previous published studies (i.e. Koehler and Wassenaar 2012 Anal Chem) that describe this type of technique for natural abundance samples (combustion + laser spectroscopy) have shown some measurable memory issues for hydrogen, at least. I suspect that labeled samples should be further affected.

p8, lines 271-273: How was this amount calculated?

p8, lines 282-283: “In the present experiment, we show that more than 70 % of the H-C bonds are broken”. Is this correct in view of the H exchangeability concern during analysis?

Figures. During the whole manuscript I missed the results of ^{13}C and ^{2}H abundances of the bulk soil and lipids during the length of the labeled and unlabeled experiment. Specially, when the correction of hydrogen exchangeability seem to be performed by measuring the labeled and unlabeled samples.

Figure 2. In the hydrogen labeling experiments performed, there are two sources of hydrogen: substrate and water. In relation to the mineralization of labeled substrates is clear to me since a starting amount of molecule (day 0) became consumed along the experiment and the labeling signature is decreased. However, for the water, it is a different story. I believe the incubators used were filled with plenty of labeled water, which means the labeled signature never is consumed or decreased. I wonder if the trend of estimated H derived from water in this figure is based on the isotopic equilibrium with the labeled water instead of an observed derivation of H from water in vivo into microbial biosynthesis. Again, as previous comment, showing the measured ^{2}H abundances over the length of experimentation could provide insights to clarify these points.

Table 3. One more noticeable thing in the table is that the results between H (% Hdfw) and C (% Cdfm) are quite consistent in lipids, which do not have exchangeable hydrogen. In the other hand, the proportion of hydrogen derived from the labeled source did not behave similarly in the bulk soil (with exchangeable H). A clear explanation on the treatment of exchangeable hydrogen can convince the reader on a differential isotopic routing of H and C.