

Interactive comment on “Land-use perturbations in ley grassland decouple the degradation of ancient soil organic matter from the storage of newly derived carbon inputs” by Marco Panettieri et al.

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We have carefully read reviewer's comments and suggestions and we have performed the necessary corrections to the manuscript.

We hope that our responses and the changes we made in our manuscript make it suitable for its publication in SOIL. Sincerely, Dr. Abad Chabbi in behalf of all the co-authors.

Revision notes:

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Reviewer comment: 1) Introduction – the organization and flow of the introduction needs to be improved. There are short paragraphs that aren't integrated, the objectives are stated before the literature is reviewed in detail. The Introduction section needs major revisions and should have improved logic flow and organization. For example: Line 47: The link of the hypotheses to the literature should be better emphasized. The current structure of the introduction doesn't make it clear how these hypotheses were derived based on research gaps in the literature. Line 55: This is a short paragraph which should be better integrated with the rest of the introduction.

Answer: The introduction has been completely reorganized as suggested by the reviewer. Some of the concepts have been clarified, and we improved the integration of all the paragraph of this section. Our first version included a first part about the needs of long-term experimentation in ley grassland rotations, then a second part focused on the technical needs to use early indicators and advanced analytical techniques. This new version presents a merged review of literature followed by the general objectives of our study. This reorganization has improved the logic flow.

Reviewer comment: Lines 66-67: NMR does not provide such information – clarify.
Answer: As suggested by the reviewer, we have reworded this sentence referring to the degradation status of SOM induced by land-use or agricultural practices, a proxy that is commonly evaluated using NMR. Reviewer comment: Line 73: Another hypothesis is stated later in the intro.

Answer: We modified the paragraph presenting our hypothesis, as suggested by the reviewer. Reviewer comment: 2) Methods – all methods seem appropriate. However, it is not justified why only LF was used. This only represents a small portion of the total soil C and analyzing this alone can be misleading. Why were the other fractions not included in some of the analyses in this study? This is a potentially significant limitation because mineral associated organic matter (MAOM) provides insight into mechanisms of stabilization and carbon storage.

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Answer: We agree that MAOM plays an important role for C stabilization and storage at long term. We have chosen LF due the characteristics explained in the introduction and discussed in the section 3.1. We added a more detailed explanation at lines 69-73.

In summary, land-use changes and crop rotations provides changes in soil C cycles in a shorter timespan than that necessary to observe changes in MAOM. Estimated turnover time of LF is shorter than that of MAOM, thus LF has been proposed in literature as an early-detection pool of C to evaluate land-use or crop rotation performances and to adapt land-use policies for C storage. A previous study on the bulk soil and aggregate fractions from the same experimental area has been published by Panettieri et al. (2017), this new study is focusing on a more reactive pool of soil C and presenting a more detailed chemical characterization.

Reviewer comment: Lines 92-93 – this information would be more useful if placed in the stable isotope section.

Answer: We moved this sentence to the stable isotope section, as suggested.

Reviewer comment: 3) Results and Discussion – the organization of this section is very poor. Many short statements with no explanation. Very little data synthesis. The authors need to improve this section for organization and clarity. They must also correct the overinterpretation of the NMR data and be weary about the detection limits of ^{13}C NMR. This section is also very hard to follow because of the many abbreviations and acronyms used. The authors should revise this entire section carefully and should separate the results and discussion so that the discussion can focus more on what the individual data sets mean when considered holistically. The current format is too fragmented and difficult to follow.

Answer: We reworded some of the sentences to clarify NMR interpretation, as suggested by the reviewer. We have included a supporting reference (Clemente et al., 2011) to justify some of our assumption. We have also reduced the use of acronyms and abbreviations. We have chosen to not separate results and discussion, but we pro-

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vided a deep reorganization of this section taking into account the suggestions from the two anonymous reviewers. We consider that this new version is less fragmented and it has considerably improved its readability. We think that, considering the high volume and the detail of presented data, a separate section for results will be long and dense and hard to follow without any data discussion associated. However, we are willing to separate this section if the reviewer and/or the editor require it necessary.

Reviewer comment: Lines 294-299 and 355 – this isn't correct, a terminal methyl group is not an indication of microbial compounds. Many plant-derived compounds have terminal methyl groups. The authors are misinterpreting the NMR Data here. The NMR data are not resolved enough to provide discreet chemical structures.

Answer: We have reworded this sentence to clarify the concept. It should be remarked that methoxyl and N-methyl resonates in different spectral regions, and that only terminal methyls bond to methylenes are resonating in the regions we considered. The ratio between terminal methyl and methylene signals has been used to show that the contribution to LF of long-chains attributed to cutins or suberins was not supported by our data. Clemente et al. (2011) used combined techniques of NMR including solution state ^1H NMR and diffusion edited ^1H NMR to demonstrate that alkyl contribution to fine fractions of soils under prairie is mostly microbial-derived. We included this reference in the manuscript and we also mentioned the possible contribution of plant-derived short aliphatic chains.

Reviewer comment: Line 345 – it is well documented that the LF is rich in O-alkyl so it is unclear what the point is here. Answer: We reworded the sentence to clarify that O-alkyl contribution was higher in samples from permanent grassland than those from the other treatments.

Reviewer comment: Line 367 – this is unclear – would changes in vegetation inputs reflect changes in SOM because there is less cutin being added to the soil?

Answer: We have reworded the sentence making a clear reference to depolymerization

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of polysaccharides.

Reviewer comment: 4) Conclusions - because of the poor organization of the R & D section, it is hard to appreciate the conclusions and how the authors made these conclusions based on the data interpretation.

Answer: The reorganized R&D section provides now more connections with the conclusions. Reviewer comment: Line 409 – all of the methods have been previously published so there is no novelty in the approach but in the insight.

Answer: We reworded this sentence as suggested.

Reviewer comment: Tables/Figures Table 3 – there are too many significant figures for the integrated NMR Data.

Answer: We think that the figures we have used are functionally to the chemometrics approach, which extracts more information from the NMR spectra. Figure 5 is functional to show how the alkyl/O-alkyl ratios (common indicators of the degradation status of SOM) are influenced by aggregate-size and land-uses. Figure 6 summarize in a PCA how the spectral regions are correlated with supplementary variables of SOM cycle, and how the PCA plan is grouping the four treatments and the five aggregate fractions on the two principal component axis. Figure 7 summarize the chemometrics approach. It highlights the differences between homologous samples from different treatments. The same results showed as “subtraction spectra” could be represented as histograms or table using the same integration procedure proposed in table 3. However, this figure allows to visually recognize patterns in the spectral regions (e.g. increases in O-alkyl or alkyl intensities) and to distinguish them from the noise/fluctuations. We are willing to reduce the figure numbers if the reviewer and/or the editor consider it necessary.

Reviewer comment: What is the level of reproducibility and detectability?

Answer: About reproducibility: we have tested field replicates for bulk soil samples and reported the standard deviations obtained for each spectral regions (as reported in

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the section 3.3) to assure the reproducibility. Working on composite samples for NMR analyses as we did for aggregates is a common approach in literature to cope with time/cost constraints, supported by the reproducibility of NMR analyses (see Diekow et al. 2005, doi: 10.1111/j.1365-2389.2005.00705.x). About detectability: NMR molecular mixing models have been developed taking advantage of other chemical variables (such as C and N contents of samples) and used to give insight on SOM composition (Nelson and Baldock 2004 <https://doi.org/10.1007/s10533-004-0076-3> is the most used molecular mixing model). We adopted a similar approach using subtraction spectra and correlation between spectral regions and field variables (total N, ¹³C isotopic signature or LF-C losses) to highlight main differences in chemical composition of LF-C from aggregates.

Reviewer comment: Typically no decimal places are used with such data due to the lack of sensitivity of ¹³C NMR.

Answer: As suggested by the reviewer, we reduced to one the decimal the data of table 3, according to the most common format found in recent literature.

Reviewer comment: Figure 6 – this figure is very busy and it is unclear what this is showing.

Answer: Figure 6 is a PCA analysis reducing to two principal components the integrations of the eight NMR spectral regions. In the 6A we represented the active variables (spectral regions) with supplementary variables related to SOM cycle (supplementary variables are not contributing to PCA calculation). In the 6B we represent the distribution of the observations in the plane described by the two principal axes. Observations from the same treatment were connected by polygonal hulls.