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Comment

Interactive comment on “Density fractions versus size separates: does physical fractionation isolate functional soil compartments?” by C. Moni et al.

C. Moni et al.

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Referee #2

The paper aims at evaluating physical fractionation procedures of soil organic matter (SOM) with regards to their ability to isolate functionally different but unique (non composite) soil organic matter pools with a homogenous decay behavior. For their evaluation of physical fractionation procedures the authors select two fractionation procedures (I) a fairly new aggregate density fractionation (ADF) and (II) a traditional and widely used particle size-density fractionation (PSDF). The two physical fractionation procedures were applied to two Cambisol soils under managed beech forest, one in France and another in Germany and samples were collected 8 resp. 12 years after application of ^{15}N enriched labelled litter. Fractions are characterized by their C- and N

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contents, C/N ratio, and $\delta^{13}\text{C}$ - and $\delta^{15}\text{N}$ -values as proxies for the degree of microbial processing. Fractionation procedures are compared and characterized through a standardized principal component analysis (PCA) to identify two independent variables that account for the majority of the data variability. To visualize dynamics of ^{15}N label incorporation, a contour map representing excess ^{15}N was applied in the plane defined by the main variables. As conclusion the authors suggest an improved 'economized' fractionation procedure that 'only retrieves meaningful fractions'. The presented dataset and especially the statistical analysis are very interesting, innovative and valuable.

1) However the overall approach is inconsistent: A clear structure is missing in the paper which should be a guide to evaluate the implications of the applications of different fractionation protocols.

We did our best to present our work in a concise manner, but have to acknowledge the fact that our efforts are not perceived as having a consistent internal structure. However, we very much appreciate the constructive criticism offered by the reviewer and are willing to generate a restructured manuscript guided by the reviewers comments

2) While some methods are introduced with dense information other methods are only mentioned (e.g. fractionation by particle size versus aggregate density fractionation).

We acknowledge some imbalance in the extent to which we recognize certain procedures. We will revise and make sure that each procedure receives due attention

3) Some parts of the introduction and the discussion are very detailed with lots of complex issues but little clearly transparent context.

We recognize the reviewers concern and offer to make sure that all items are put into context.

4) The intention why the authors selected two specific 'standard methods' for their examination despite they addressed in the introduction methodical limits do not become

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clear.

Our choice of fractionation procedures was governed by the intention to (i) represent at least two different physical separation principles and to (ii) achieve maximum physical diversity among individual fractions (i.e. single mineral particles, particulate organic matter, fine fractions, and aggregates). The benefit of having many physically different fractions is seen in an enhanced ability to generalize results to other fractionation procedures not tested in this reserach. We will highlight this intention in a revised version.

5)Please state more clearly your objectives and your hypotheses! The points 'lack of knowledge', 'hypothesis' and 'new findings' just do not come out clearly enough.

We regret that our goals, objectives and hypotheses appear to have remained obscure to the reviewer. We are confident that a revised internal structure of the manuscript will help us to improve and add emphasis here.

6)Shortening and focusing of the text would help greatly.

As we restructure the manuscript, we will seek to cut text where possible

7)The application of the two standard methods led to already well known results (Chapter 3.1 to 3.5). Chapter 3.2 demonstrates that the obtained fractions are not pure and statistical analysis was applied to evaluate 'mixed' fractions. This part is interesting, new and informative and the potential of such statistical analysis and possible applications could be better 8)highlighted and discussed in the manuscript!

We respectfully maintain that so far, no direct comparison between stable isotope signatures in density versus size fractions has been published. For that reason, we do claim that our results are novel in a sense that they show for the first time how physical fractions obtained by different separation principles differ in their ability to represent features such as microbial processing. However, we acknowledge that this fact can be emphasized. We will also offer more descriptive detail on the evaluation of composite fractions.

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9) Variables used for PCA are proxies for the degree of microbial processing and I regret that no unique indicators could be used.

We agree, but at this point have to ask the reviewer for patience. There is work in progress that we eventually hope to present in a future publication

10) Further, following the dynamic of ^{15}N label incorporation basing on the relative enrichment ($E^{15}\text{N}$) can be biased in the field by 'dilution' effects. The dynamic should instead be based on the mass-losses or mass increases of ^{15}N . The discussion and the evaluation should take these issues into account and should be done more cautiously.

We fully agree with the reviewers comment on dilution effects but are not convinced that mass – based parameters would have served our purpose better. We will incorporate a brief discussion of this issue in the upcoming revisio, as requested by the reviewer.

11) The proposed new fractionation procedure seems promising but no generalization should be made by the examination because only one soil type (Cambisol) was used and serious discussions in view on different soil properties (e.g. different textures, different carbonate/dolomite concentrations...) and of comparable examinations in the literature are missing. Density cut-offs and intensities of dispersions should be tested and discussed on a broader set of soils.

The proposed new scheme has the characteristic of an automatic consequence of our work – we found that some fractions isolated by the procedures used were not at all meaningful. As a result, we do not recommend that the audience should bother with them. It was not our intention to claim that our suggestion would be applicable to all soil orders without further testing and evaluation. We will emphasize this point in the revision

12) Specific comments: In Chapter 1.2, Chapter 1.3 and Chapter 2 important details as well as references are missing that are necessary for a critical evaluation: Chapter

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1.2: Description of advantages/disadvantages of the fractionation procedure by size: please add references!

Some of these points are deduced, some are taken from the literature. We will make it clearer where we deduce and where we quote

13)Chapter 1.3: Generally: For each method add a statement with regards to the potential usefulness of the method.

Not a problem, will be implemented in the revision

14)Aggregate density fractionation: Methodological points of critique are not mentioned and discussed. E.g. medium densities do not separate 'true aggregates' but also light organo-mineral complexes and/or very small-sized particles.

This will be implemented in the revision as requested

15)Chapter 1.4: Please state more clearly your objectives. Why did you select the two fractionation procedures (although you described in detail their limitation)? What do you intend?

Will be addressed in the revision. Please compare explanation above (items 4 and 5)

16)Chapter 2.1: "highly enriched ^{15}N litter": Please tell in more detail. Please summarize the results by Hatton et al. (2012) that are necessary to understand the experiment.

This will be implemented in the revision as requested

17) Chapter 2.2.1: "Assuming an average particle size density of 2.44 g cm^{-3} ": Please explain in more detail and add references.

This will be implemented in the revision as requested

18) Describe in more detail and more objectively how fractions coarser than $>6\mu\text{m}$ were separated: "repeatedly soil fractions were gently swirled in..." : How often and how long and how did you shake the solution? How much time was needed for sedi-

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mentation. . .

Best way to do this would be to add visuals (video). Would this be an option for the journal ?

19) Chapter 2.2.2: Please add a short description of the method proposed by Sollins et al (2006) and the usefulness of the method. What did you intend?

This will be implemented in the revision as requested

Important literature is missing. E.g.: Kaiser, K., Guggenberger, G., 2007. Distribution of hydrous aluminium and iron compounds over density fractions depends on organic matter load and ultrasonic dispersion. *Geoderma*, 140: 140-146. Cerli, C., Celi, L., Kalbitz, K., Guggenberger, G. and Kaiser, K., 2012. Separation of light and heavy organic matter fractions in soil – Testing for proper density cut-off and dispersion level. *Geoderma*, 170(0): 403-416.

We will read (again) and cite when and where appropriate

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