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Interactive comment on "Are researchers following best storage practices for measuring soil biochemical properties?" *by* Jennifer M. Rhymes et al.

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Comment by Hanna Frick & Else K. Bünemann (with contributions from Maike Krauss, Andreas Fliessbach)

As a group of researchers from the Soil Science Department of the Research Institute of Organic Agriculture (FiBL), Switzerland, we discussed the manuscript entitled "Are researchers following best storage practices for measuring soil biochemical properties?" by Rhymes at al. 2020.

Rhymes et al. 2020 raise the discussion on an important topic that concerns the whole

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soil science community. We would like to acknowledge the authors′ enormous work in a comprehensive and valuable case study on best practice storage conditions for soil samples and soil extracts for various commonly investigated biochemical parameters (with almost 2000 extractions performed). We highly appreciate their initiative in raising awareness on this vital, but often neglected topic and hope that their contribution will spark further work and exchange among soil scientists.

However, in our opinion some important aspects were not considered adequately and we have the following suggestions for improvement:

1) The data on which Rhymes et al. base their guidance should be provided in the main manuscript rather than the supplementary information (SI). While the authors themselves claim that "[. . .] optimal storage conditions will vary across different soils and ecosystems" (Line 75), but also between top- and subsoil, as shown by their own case study, they come to very generalized recommendations on best practice storage in Table 2, which we find contradictory. To us it is not quite clear how the authors come to their recommendations, or at least some differentiation is lacking. For instance, in Figures S4a and S5a, storing frozen extracts up to 430 days seems tolerable for both MBC (only topsoil) and MBN (both top- and subsoil), but in Table 2 freezing extracts for assessment of microbial biomass is indicated as completely inappropriate.

2) The discussion of changes upon storage should be further elaborated and put in context with existing literature (e.g. the literature reviewed in Table 1). For example, Stenberg et al. (1998) suggest that soils can be stored frozen for up to 13 months for assessing microbial biomass, while Rhymes et al. recommend not to freeze soil at all for any kind of biochemical analysis they considered in their manuscript. How would the authors explain these differences? In Line 130, Rhymes et al. speculate about microbial processes as the main driver of changes in stored soils or extracts and they suggest storing samples under conditions which suppress microbial activity completely. Given the major changes still happening in frozen extracts over time for NO3 (Figure S3a), do the authors suggest that freezing is not suppressing microbial

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activity sufficiently? Could there be other mechanisms responsible for this trend? The discussion currently provided in the SI should be moved to the main manuscript in order to increase its visibility to the scientific community.

3) The importance of the underlying research questions is neglected: The authors only look into relative changes in the measured parameters in comparison to freshly extracted and immediately analyzed samples. However, many studies aim at investigating relative differences between treatments rather than obtaining absolute data on fresh samples. In fact, appropriate storage conditions are not only part of the method, but also depend strongly on the research question. In many cases, standardized pretreatments (for example pre-incubation of soil after refrigerated storage for microbial N and C), freezing of all samples before extraction etc. might produce smaller errors than immediate extractions, where differences upon sample collection, transport, outside temperature upon sampling etc. would arguably cause bigger effects than the storage treatment. With this regard, especially the change in the measured parameters upon prolonged duration of storage is relevant. For instance, Rhymes et al. consider freezing of soil or extract for analysis of NH4 inappropriate (Table 2 or Figure S3b), however, changes here seem to appear immediately upon freezing, with marginal changes thereafter (Figure S3b). For studies only interested in relative differences between treatments or sites, freezing thus would be a tolerable storage method. Again, we think that the recommendations should be more differentiated and take potential research questions into account.

4) From our own experience, but also highlighted by the results of the survey which Rhymes et al. conducted amongst different laboratories (note that the documentation on how the survey was conducted could be expanded), storage of both soil and soil extracts are common practice. This is owed to the mere impossibility to collect, extract and analyze samples in one day, especially with high sample numbers or when sample collection has to be conducted at large spatial distance to the lab. In this context, we find their conclusion on “appropriate” or “inappropriate” storage too general. How

about defining an acceptable relative error, e.g. by handling the samples in one way or the other? Furthermore, as indicated above, relative errors occurring immediately (e.g. upon freezing or un-freezing) should be distinguished from continued changes upon prolonged storage.

5) With their study, Rhymes et al. made an important point on the effect of storage conditions, but we miss the broader picture. The discussion should expand also on other aspects potentially compromising the integrity of soil samples, such as sampling procedure, transport, pre-treatments or handling of the samples in the laboratory. We believe that the whole soil science community should put more effort into defining common standards and evaluating potential errors during the whole procedure from sample collection, transportation and storage until analysis. Comparing the effect of storage conditions with the effects of these other aspects would help to identify sources of major errors and design experiments accordingly.

6) If each group has to carry out their own pilot studies and resulting storage conditions will vary substantially, then meta-analyses will become even more difficult than they are now. Besides, the recommendations for such pilot studies would need to be really concise, e.g. how many time points would need to be analyzed? It would be important to learn as much as possible from the experiment conducted by Rhymes et al. As an alternative to pilot studies, why not put an effort into identifying suitable reference materials that can be included in each study?

In addition to these general thoughts, here are some more detailed comments:

Sampling procedure and soil sample preparation

- While we understand the reasoning behind their sampling approach (topsoil sampled three weeks after sampling the subsoil), in most of our experiments this is simply not an option, e.g. due to distant sampling locations and the importance of a uniform sampling time point.

- Soil samples were taken in June. Would results be different if soils had been sampled in winter or at a different initial water content? Generally speaking, the effect of seasonality should be discussed.

- Apparently, Rhymes et al. use “field replicates” for their extractions (SI Line 43ff): Soil was sampled from five locations (transect over the field with 10m distance between plots) in 0.5 x 0.5 m pits. These replicates were later on used for the extraction/different storage treatments. This sampling approach explains the high data variability upon the individual time points and storage conditions and should have been discussed by the authors.

- The time of sieving/homogenization was not investigated, since Rhymes et al. sieved all soil samples on the day after sample collection and stored all the soils sieved. Would the results have been different if soils had been sieved only after storage, immediately before extraction?

Extraction procedure and handling of extracts

- SI Line 71: For K2SO4 extraction, no blanks were performed. While this seems valid for the calculation of microbial C and microbial N as difference between fumigated and non-fumigated extracts, we find this problematic for reporting values on total C and total N in both fumigated and non-fumigated extracts, which were not corrected for blanks (compare Figures S4 b, c and Figure S5 b, c)

- The molarities of extractants (K2SO4, KCl) are not reported throughout the whole manuscript.

- Scaling of extractions procedure: Authors report that 5 g of moist soil were extracted. This is a very low amount considering any potential inhomogeneity in the soil. Due to the high number of replicates (n=5) this might be acceptable. Additionally, soil moisture content (e.g. between top- and subsoil) was ignored upon extraction, which might lead to differences in the soil-to-solution ratio. Equal amounts of dry soil equivalents should

be used for a standardized extraction procedure.

- Scaling could also be added as another point to consider for a pilot study within Table 3 (extraction methods; recommendation: do not up-/down-scale the used amounts but use the same amounts as planned for the main experiment).

- Freezing and un-freezing procedures were not investigated as further factors. From our experience, it makes a difference in which position extracts are frozen (e.g. vertical or horizontal placement of tubes) and under which conditions extracts or soils samples are thawed (e.g. thawing soils over night at 4°C or extracting frozen soil immediately with the solution).

Statistics/Figures/Data presentation

- SI Line 104: Why did the authors use a plot digitizer to extract numeric data from their own plots?

- Figure S1b: In the figure caption, authors indicate that there was a technical problem with the DON measurement on the last time point (Day 430) and thus, data should not have been included. However, in the figure there a data points also for this sampling time.

- For some of the analyzed parameters, the replicates show a very high data variability. However, this seems not always represented in the confidence interval displayed (e.g. Figure S3 a: NO3 values for frozen extracts vary widely, while the confidence interval seems to be very small).

- In Table 3, authors recommend to use twice the number of replicates for the baseline (freshly extracted and analyzed samples). However, for their own case study they did not follow this recommendation or at least did not report it.

Technical comments:

- Typo in Table 1: Plant available N, reference “Jones and Willett 2006”, under storage

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methods explored it should probably be -18°C

- Line 60: Wording is misleading. Stenberg et al. 1998 also sieved the soil prior to storing it at different temperatures.

- Table 2: There seems to be a mistake in the table header. We do not see any red or green squares. We assume that the information given below the table ("Dark grey denotes inappropriate storage method and light grey appropriate.") gives the same information?

- Line 135: Figure 1 should only have a figure caption below, but not additionally above.

- Table S2: Table header is missing.

STENBERG, B., JOHANSSON, M., PELL, M., SJÖDAHL-SVENSSON, K., STENSTRÖM, J. & TORSTENSSON, L. 1998. Microbial biomass and activities in soil as affected by frozen and cold storage. Soil Biology and Biochemistry, 30, 393-402.

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