In this short communication the authors address the lack of evidence-based recommendations for storage of soil samples and extracts prior to analysis of organic and inorganic carbon (C) and nitrogen (N) and microbial biomass C and N. Based on literature and an online survey, the authors demonstrate that samples and extracts are stored in a multitude of ways. They further provide a case study in which they demonstrate the effects of sample and extract storage on measurements of organic, inorganic and microbial C and N, further stressing the need for standardisation of protocols.

The manuscript offers recommendations for sample and extract storage for the investigated methods, a flow-chart to guide researchers deciding on the most appropriate storage approach for their experiments and recommendations for reporting of the methods adopted for scientific publications. The results of the case study are a welcome reference for future research. In particular, the case study demonstrate that storage methods do not affect samples of different soil types (top vs subsoil) equally. This finding goes against the common assumption that storage affect all sample types similarly.

This short communication is relevant for the international science community, and fits well in the scope of SOIL. The introduction outlines the motivation of the objectives clearly, and the necessary details of the case study methodology and results are given in the supplementary material. The text is well written and pleasant to read. I have a few suggestions for corrections. Tables and figure are mostly supportive (particularly table 2). I have some suggestions to further clarify the text in tables 1 and 3. Also, I wonder whether the message the authors want to convey in section 4 with Table 4 isn't better served by integrating its information into the text. Lastly, I would ask the authors to consider including Fig S6 into the main text. I outline my general and minor comments in more detail below.

Thank you very much for the positive comments and useful suggestions. We have detailed how we will address all the suggestions and recommendations proposed, which we believe will improve our manuscript. See details below.

General comments: The authors conducted an online survey and reflect on the outcome in L65 and further. Adding few lines about the nature of the survey and how representative the responses are would increase the value of its outcome in the authors argumentation.

As recommended, we will include information on the survey data collection. The survey was conducted anonymously on Google surveys and promoted through Twitter a social media platform. A total of 68 participants provided information on how they typically store their soil and/or extract samples. Unfortunately, we do not have any survey participant demographics (e.g. types of institution, geography, positions of those who answered). Although this would have provided valuable insight as to who carries out specific storage methods and the representativeness of the responses, we decided that responses would remain anonymous as we felt that some researchers may not want to disclose how long samples may be left in storage prior to analysis.

We will add the survey questions below to supplementary materials as detailed below:

1) ***Realistically, how do you process your samples prior to carbon and nitrogen analysis (KCl, K_2SO_4, H_2O)?***
    i) ***Extract and run all my samples immediately***
    ii) ***Extract immediately and store the extract until analysis***
    iii) ***Store the soil and carry out extraction for immediate analysis***
    iv) ***Store the soil and store the extract***
2) ***At what temperature do you generally store your extracts?***
    i) ***4°C***

ii) -20°C
iii) -80°C
iv) Room temperature
v) I do not store extracts prior to analysis
vi) Other:

3) At what temperature do you generally store your soil?
i) 4°C
ii) -20°C
iii) -80°C
iv) Room temperature
v) I do not store soil prior to analysis
vi) Other:

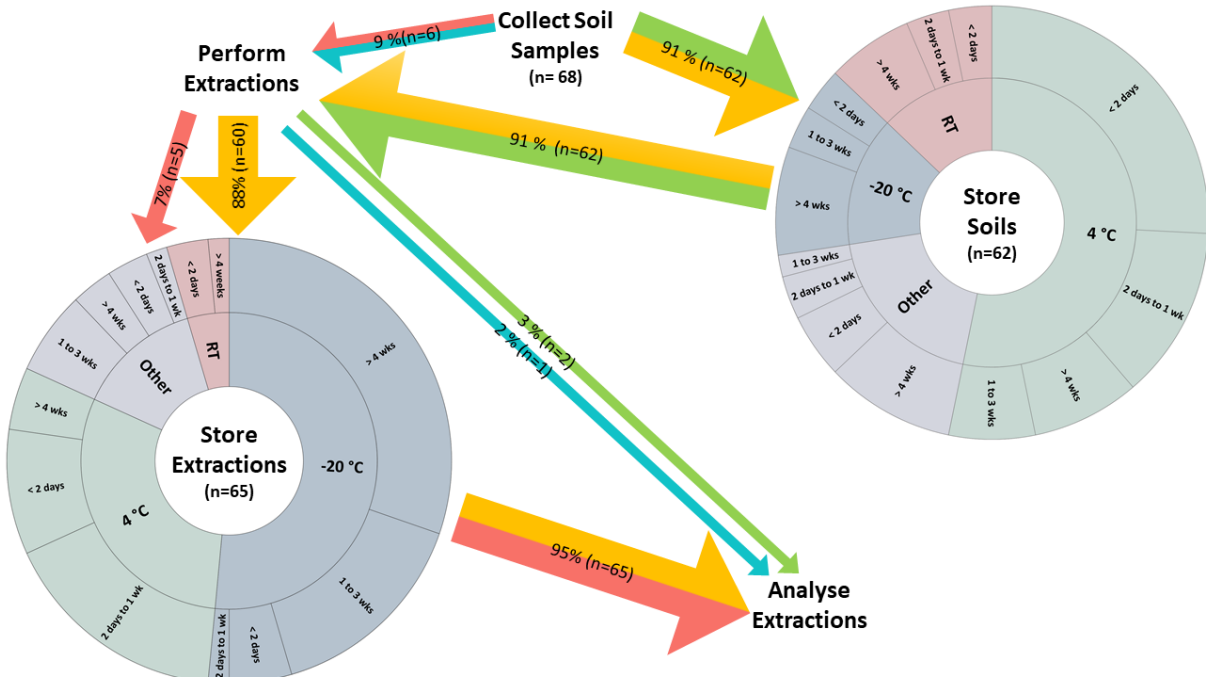
4) How long do you typically store your soils for prior to extraction?
i) 2 days or less
ii) 2 days - 1 week
iii) 1 - 3 weeks
iv) 3 weeks - 2 months
v) Longer than 2 months
vi) I do not store soil prior to analysis

5) How long do you typically store your extracts for prior to analysis?
i) 2 days or less
ii) 2 days - 1 week
iii) 1 - 3 weeks
iv) 3 weeks - 2 months
v) Longer than 2 months
vi) I do not store extracts prior to analysis

6) Any additional comments?

Also, inclusion of Figure S6 into the introduction would aid the reader follow the narrative, or at least place it first in the Supplementary materials. For Figure S6, I recommend the authors to clarify the meaning of the colours, explain the abbreviation of RT, and consider scaling the size of the arrows to the % of cases.

We agree that we should move Fig S6 to the start of the supplementary material. We will also clarify in the figure caption what the colours mean and scale the arrows, as requested; please see the new figure below. The new figure caption will read: "Flow chart indicating the results of an online survey to determine how and for how long people store soils and/or extracts prior to analysis. Room temperature is abbreviated as RT. Survey date: December 2018 – June 2019. The proportion of people that followed each methodological step is indicated as 'n' in each arrow, which are also scaled in size depending on 'n'. The arrow colours correspond to the differences in soil sample storage, processing and extract storage prior to analysis. Pink: soil samples are extracted immediately after collection, extracts are stored for later analysis. Blue: Neither soils nor extracts are stored. Green: soils are stored after collection, but extracts are analysed immediately after extraction. Yellow: both soils and extracts are stored."



I miss the mentioning of other storage methods than those considered by the authors in their case study. L73 states the authors considered widely used storage methods, but it does not become clear to me how the selected methods compare to alternatives, for example drying. I recognize that a full evaluation is beyond the scope of this short communication yet adding a few examples could bring the proposed manuscript into a broader perspective. For example, storage and the achievability of soil samples are important considerations when choosing indicators for monitoring efforts (Ritz K, Black HIJ, Campbell CD, et al (2009) Ecol Indic 9:1212–1221. https://doi.org/10.1016/j.ecolind.2009.02.009).

In response to the reviewer's comment, we will also expand on other storage alternatives in the introduction section to broaden the perspective of this short communication. We will add the following text to line 33 "In these cases, it is common practice to store samples for future analysis of which there are a broad range of storage practices. These include freeze drying, air drying, freezing and refrigerating samples and are typically chosen dependant on the analysis in question and time in which analysis can take place."

We will also add a short discussion as to why we chose to explore refrigeration and freezing treatments. From line 69, we will add the following text: "In our case study we chose to explore refrigerating and freezing storage practices in comparison to other storage methods (e.g. air drying or freeze drying) because there is significant evidence to suggest that those methods are unsuitable for the variables we measure. For example, air drying soils has a strong effect on C and N pools, probably due to microbial death and nutrient release upon drying and rewetting (Jones and Willett, 2006; Kaiser et al., 2001; Li et al., 2012; Rolston and Liss, 1989). Additionally, freeze-drying is also known to have a strong effect on nutrient pools, as the chemical, physical, and physiological stresses inflicted by freeze-drying severely injure or kill most soil microbes, releasing the microbial compounds into the soil (Islam et al., 1997).

We would also like to highlight that our chosen storage treatments are commonly practiced as highlighted by the survey: 66% of participants store their soils at 4°C or -20°C whilst 87% of participants store their extracts at 4°C or -20°C.

While sections 2 and 3 give helpful directions to soil scientists, I find section 4 less strong. Table 4 presents best reporting practices, yet the section does not mention why the listed requirements are important, nor gives a motivation for providing these recommendations. Also, I would recommend the authors to remove table 4, and instead list the four reporting recommendations in the text in section 4. To convey the best reporting practice, I don't think it is necessary to provide examples of poor reporting and giving examples of good reporting are easier to read when listed in the text.

We agree and will remove table 4 and integrate some of the information in the table into the text. We will also highlight why it is so important to report storage methods in section 4. This will include data that can later be used for meta-analysis.

Minor issues:

L43: "that have considered these have taken into account few..." makes it easier to read

This will be made clearer: "In many comparative studies exploring the impacts from methodological factors overlook soil and/or extract storage temperatures and duration, and those that have considered these, have taken into account few storage possibilities (Table 1)".

Table 1: Column 6 "storage methods explores", remove the word 'only' in the listed examples.

We will remove only throughout the table.

Table 1: Yolo loam: what soil type reference was used, e.g. WRB soil types?

These are Typic Xerorthents, USDA classification. https://soilseries.sc.egov.usda.gov/OSD_Docs/Y/YOLO.html

https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs142p2_051232.pdf

Table 1: H2O – Rolson and Liss: at what temperatures were the soils stored frozen?

We will amend this and add -10 °C

Table 1: Plant available N – Soil Type: what is the difference between not applicable and not provided?

We use the term "not applicable" for general recommendations that are made in the literature but are not based on comparative studies or investigations. We use the term "not provided" for comparative studies that do not describe the soils explored for different storage methods. We will make this clear in the table caption, which will be revised to read: "Summary of different recommendations for storage of soil or extract samples to measure soil nutrients found in the literature. This summary is non-exhaustive. The term not applicable under soil type refers to studies that were not based on comparative studies and therefore were not carried out on a soil type. The term not provided for refers to comparative studies that do not describe the soils explored in the methods"

Table 1: Plant available N – Jones & Willett 2006: can the text on soil type be shortened? F.e. "Unclear. All samples taken from temperate, oceanic locations";

We are happy to make this change.

Table 1: Same row, last column: unclear what is meant with broad recommendation. What makes that a limitation?

We will make this clearer. The recommendation made in the referenced publication states "store the extract for days". It is unclear how many days. We will change this to "Vague recommendations made for extract storage length which could be open to different interpretation".

L129: “suppress”

We will change this to “halt” as suggested by reviewer S1.

Table 3: Recommendation listed for Storage Methods is not formulated as a recommendation but reads like an observation.

We agree with this point. As we will include more details on alternative storage methods in the introduction, we will remove this row from the Table 3.

Table 3: Replicates-Heterogeneity: authors recommend 5 replicates, but it is unclear on which this is based. Statistically, number of required replicates depends on the variation within the group/treatment.

We take this onboard and would like to address this issue by amending our recommendation in Table 3 to read: “Generally, we recommend as many replicates as one can afford to have, but recommend no fewer than 4 as suggested by Jones and Willet* (2006). *For more guidance on choosing the number of replicates, we advise researchers to utilise the sample size calculator formula from Cochran and Cox (1957), p. 20. ”.

L155: “in the literature are”

This will be corrected.

Supplementary material: Table S2 is strangely outlined in the text and stands separately from its caption.

We will ensure the position of the table caption is above the table

Statistical analyses: how were normality and homogeneity assumptions checked?

We will include this information to our statistical analysis. “Normality and homoscedasticity of the data were first checked using Anderson Darling and Levene’s tests respectively.”

L99: unclear which variables were log transformed before analysis. Is this natural logarithm or Log10?

We will include this information in the statistical analysis section of the manuscript. “All variables were subjected to natural log transformation except for DOC which was not transformed.”

Fig S6: see in general comments

We will move Fig S6 to the start of the supplementary.

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