

The authors of this manuscript investigate the effects of storage method and duration on soil dissolved organic carbon (C) and nitrogen (N), and inorganic N. They identified common storage parameters through a literature survey and an online survey, and then tested how these common storage methods affected recovery of extractable dissolved C and N. Based on this experiment they report recommendations for storage of soil samples. This manuscript is important to Soil science community and fits well within the scope of SOIL. The manuscript highlights the of standardization in storage parameters when measuring dissolved C and N. Furthermore, they found that different soils (subsoil vs topsoil) were affected differently by storage method. This manuscript was well written and pleasant to review. My major recommendations are related to the presentation of the case study data, and discussion of Soil storage methods.

***Thank you very much for your response. We have addressed all of your recommendations, which we believe will strengthen the manuscript. We provide a detailed response to each recommendation below.***

***We would like to clarify that the survey was actually carried out after our experiment. The purpose for the survey was to highlight the fact that researches do store soil samples and soil extracts, even though this information is usually not added into their methods. We also aimed to identify the proportion of researchers that carried out different soil storage methods.***

General comments:

I believe the manuscript as a whole would benefit from including the major findings from the case study into the main text. These results are currently included in the Results section of the supplement. Furthermore, when included in the main text, the major findings of the case study should be briefly summarized in the abstract. Section 1 clearly states the importance of the standardizing soil storage methods.

Section 2 The Case Study is the strongest evidence that there is a problem with non-standardized storage methods. Therefore, the results of the study should be included in the manuscript. The figures could remain supplementary since Table 2 summarizes the results. However, the Results should be moved to the main text with appropriate figure citations included in text.

***As suggested, we will expand Section 2 by moving the results section from the supplementary into the main manuscript, this will include line 107 to line 172. These lines include the description of the results obtained for DOC and DON measurements in water extracts (lines 107-135), extractable ammonia and nitrate in KCl extracts (lines 136-149), and microbial biomass carbon and nitrogen in K<sub>2</sub>SO<sub>4</sub> extracts (lines 150-172). We have kept the figures in the supplementary material and will now refer to them in the main manuscript. The amended abstract that includes our case study findings will read:***

***“It is widely accepted that the measurement of organic and inorganic forms of carbon (C) and nitrogen (N) in soils should be performed on fresh extracts taken from fresh soil samples. However, this is often not possible, and it is common practice to store samples (soils and/or extracts), despite a lack of guidance on best practice. We utilised a case study on a temperate grassland soil taken from different depths to demonstrate how differences in soil and/or soil extract storage (4 °C or -20 °C) can compromise sample integrity for the quantification of soil dissolved organic C and N (DOC and DON), extractable inorganic nitrogen (NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup>), and microbial biomass C and N (MBC and MBN). The appropriateness of different storage treatments varied between topsoils and subsoils, highlighting the need to consider appropriate storage methods based on soil depth and soil properties. In general, we found that storing soils and extracts by freezing at -20 °C was least***

***effective at maintaining measured values of fresh material, whilst refrigerating (4 °C) soils for less than a week for DOC/DON, up to a year for MBC/MBN, and to refrigerate soil extracts for less than a week for NH<sub>4</sub><sup>+</sup>/NO<sub>3</sub><sup>-</sup> did not jeopardise sample integrity. We discuss and provide the appropriate tools to ensure researchers consider best storage practice methods when designing and organising ecological research involving assessments of soil properties related to C and N cycling. We encourage researchers to use standardised methods where possible and to report their storage treatment (i.e. temperature, duration) when publishing findings on aspects of soil and ecosystem functioning. In the absence of published storage recommendations for a given soil type, we encourage researchers to conduct a pilot study and publish their findings.”***

Line 93. Were the statistics performed in R? This should be stated and if the mixed effects models were performed using an R package that package should be cited. It appears they are cited in the supplement. That should be moved to the main text.

***As suggested, we will include this information in the main manuscript and reference the packages accordingly. Line 89 will start as: “All statistical analyses were carried out in R Version 3.6.1 (R Core Team, 2019).” And in line 93: “Mixed-effects models were performed for each measured variable with lme4 package (Bates et al., 2018) to test the effects of fixed factors ...”***

Line 111-115 - Table 2: There appears to be a discontinuity between the table legend and the table itself. The table legend specifies that Red and Green squares, while the table has dark and light grey squares. It appears to be properly labelled in the note at the bottom of the table.

Line 112-113 - I am not following the statement from the legend, “Green denotes appropriate storage method with additional recommendations for storage length”. Where are the additional recommendations? If they are in the main text I believe that should be specified. Line 113

***Thank you for highlighting this mistake. The legend and text will be corrected to read: “Table 2. Storage method recommendations for both temperate topsoil and subsoil. Dark grey denotes inappropriate storage methods for specific analysis. Light grey denotes appropriate storage method, where appropriate storage length is annotated. Where storage length is annotated as 430 days we are unable to advise storage length beyond this due to the length of the experiment.”***

Line 113 - I am also confused by this statement “Where we do not specify, stored samples did not differ from fresh samples through the entire experiment, 430 days.” The only areas not specified are the dark grey boxes, which I understand to indicate an inappropriate storage method. Please clarify.

***We have removed this statement. We mistakenly didn’t update the table caption accordingly when we changed the cells from being empty to then read <430 days.***

Additionally, if they did not differ during that time, then the sign should be greater than or equal to, or simply stated that recommendations beyond 430 days cannot be made due to the length of the experiment.

***We will correct this. We will remove the < symbol from the table and state in the legend that we cannot advise storage beyond 430 day due to the length of our experiment (Please see amended caption above).***

Section 3 and 4 are strong. However, it appears to be implied that these recommendations are specific to studies comparing different soil, e.g. topsoil vs subsoil. Based on the study and the recommendations, it does not appear to apply to researchers carrying out an ecological study using a single soil that has been experimentally manipulated. If the authors agree with my conclusion then

they should state that their recommendations do not apply in the circumstance. If they do not, then I they should provide a strong argument for why not.

***We appreciate this remark and want to clarify that these recommendations would apply in the same way as if a study were using one soil type, because the accuracy of the measurements will still be affected by storage method. We will include an opening statement in section 3 to address this as follows: “The case study findings highlight how integral it is to consider best storage practice for soil analysis in any study/experiment. These include studies exploring one or more soil type, site location and/or treatment manipulation, as the accuracy of the measurements can still be affected by storage method despite samples coming from one soil type, location or treatment. Please, see further details on this issue in the response to the short comments SC1, issue 3, where we give a specific example and discuss why freezing is not a tolerable method for storage when studies are only looking at relative differences between treatments and/or site.***

Section 5 The authors should also address experimental manipulations of single soil types here as well.

***This point will be addressed in section 5. The table will be incorporated into text. We will clarify that one must still consider best storage practice when only working with one soil type.***

Technical notes

Line 19 space needed between “andNO3”

***This will be corrected.***

Line 31 I think this citation may be wrong in the citation manager because it does not have the author’s name here or at the beginning of the citation in the references.

***We have checked and this reference is correct. ISO report standardised methods for laboratory practices and are usually referenced without an author name.***

Line 60 Add a space after the 4 and before the degree symbol

***This will be corrected.***

Table 1 Formatting to prevent last letter of some words moving to next line Line 180, I don’t think the parentheses are necessary around 2020

***This will be corrected.***

### **References**

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G., Green, P., 2018. Package ‘lme4.’ Version 1, 17.

Team, R.C., 2019. R version 3.6.1 (2019-07-05) -- “Action of the Toes” Copyright (C) 2019 Platform: