Reply to Reviewer 1

In the following, please find the corrections and comments to the referee's response.

General comments

This inter-laboratory comparison study presents very significant findings regarding the performance of cryogenic water extraction (CWE) systems for soil water stable isotope analysis. The paper is well written with clear findings and illustrations. The lead and senior authors have developed and researched CWE system for several years and have previously published several papers on these systems (e.g. Orlowski et al. 2013, 2015, 2016). The present study represents a welcome initiative to help improve analytical techniques. The comparison study was well designed with the emphasis on documenting the difference between the known isotopic composition of a supplied water sample and the extracted isotopic composition of soil water derived from the known water by using it to wet dry soils. The key finding is the surprisingly large difference in performance of CWE systems in different laboratories (Fig. 3 and 4). A few laboratories performed relatively well but none were acceptable (as per the study criteria) for all soils, water contents and isotope systems. The fact that most laboratories performed very poorly for one or both soils using a system that has been regarded as the main stay of soil (and plant) water isotope analysis is a disturbing conclusion (the authors note their dismay). ! What are the consequences for the reliability of the numerous previous studies relying on these and similar laboratories? Were studies conducted without the rigorous quality control (recovery of known water isotopic composition) carried out in the present study? These questions should be noted (if not answered) in the discussion. The interlaboratory comparison confirmed the influence of many factors that affect accuracy as documented in previous publications. However, the lack of systematic relationships between isotopic recovery of soil waters and CWE parameters prevented clear conclusions from being drawn regarding which future steps can be taken to improve performance. This suggest that a complex interaction of many factors including soil type, temperature, vacuum etc. influence CWE results. These may also include the specific design and operation of each CWE system. These findings are also highly significant in light of the Orlowski et al 2016 study (Hydrological Processes: Intercomparison of soil pore water extraction methods for stable isotope analysis). Natalie Orlowski, Dyan L. Pratt and Jeffrey J. McDonnell which compared five different techniques for analysing soil water isotope composition. It appears that the differences in accuracy of these five techniques were no larger than the difference in accuracy between the sixteen CWE systems presented in the present study. ! This raises the possibility that the success of any of these techniques may depend more on the specific understanding, design and settings/operation of each technique than an inherent superiority of one technique over another. This aspect should be added to the discussion.

General comments

Response: We thank Niels Munksgaard for taking the time to review our manuscript and providing this generally positive feedback.

Most past studies that applied CWE did not or barely provide any information on the applied extraction parameters and did not carry out any sort of quality control of the system's reliability (as per Orlowski et al. (2013)) and the obtained CWE isotope data (as per Gaj et al. (2017)). Therefore, possible fractionation effects associated with CWE remain unknown for most past studies. As we already argued in our recent paper (Orlowski et al., 2018), when CWE data is used to calculate plant's

water source, errors could be quite large and lead to misinterpretations of the role different plant species play in hydrologic processes at the ecosystem or larger scales (Zhao et al., 2016). However, in order to understand from which soil water source plants take up their water, we need to have a sound understanding of the interactions between water (mobile and higher tension water) and the overall soil compartment. Current lab-based water extraction techniques (not only CWE) remain one of the biggest challenges in achieving this goal (Orlowski et al., 2018). We will add this argumentation to the revised manuscript version.

We will further add the following to the discussion section of the revised manuscript: “In the light of our experience with other soil water extraction techniques (Orlowski et al., 2016b), we argue that the success of any of these methods may depend more on the specific understanding and operation leading to internal reproducibility of each individual technique’s results than an inherent superiority of one technique over another.”

Specific comments

1. P5 L20: I understand that choices had to be made but it should be acknowledged that the drying and rewetting steps may have influenced the outcomes if not performed the same way - were instructions on these steps included?

Response: We already discussed this point in our previous version of the manuscript: “Again, it should be stressed here that for our intercomparison soil samples were oven-dried twice (before and after shipment) prior to any rewetting and labs were advised to store the dried samples in a desiccation chamber until use…sample preparation might have played its role, when it comes to discrepancies in lab’s results… Remoistening of oven-dried soil samples might be a general problem of such spiking experiments….However, so far, regular oven-drying of soils is standard practice (Koeniger et al., 2011) for such rewetting experiments in the literature.”

2. P5 L27-32: I can’t locate the data from this reliability test, were all labs successful in this test?

Response: We did not include these results in our manuscript since not every lab provided the full set of data. We therefore picked some examples.

3. P6 L14: “alternating fashion” - it is unclear exactly how this step was carried out

Response: We will revise the sentence as follows: “Soil and DI water were added alternately.”

4. P6 L14: How much soil was loaded by each lab (both using their own method and the prescribed method)? The questionnaire asked this question, but the information doesn’t seem to be presented. Is it possible that soil inhomogeneity was a factor if a lab used small amounts?

Response: We will add this information to Table 1 and the respective results section: “In relation to the amount of used sample material, most labs either introduced 10 or 20 g to their system no matter the extraction approach (I or II), soil type or WC. Only labs 11 and 13 chose different weights with respect to the WC, e.g., 10 g for the higher WC (20%) and 20 g for 8% WC for extraction approach I.”

5. P13 L27: Incomplete drying before wetting may also have led to >100% recovery during CWE

Response: We included this point in the previous manuscript version: “Again, it should be stressed here that for our intercomparison soil samples were oven-dried twice (before and after shipment) prior to any rewetting and labs were advised to store the dried samples in a desiccation chamber until use.” And further: “However, oven-drying was performed at an intermediate temperature (105°C for 48h) and not under vacuum as per Savin and Epstein (1970) and different indoor laboratory ‘climatic conditions’ at the participating laboratories were observed. Thus, it might be possible that not all of the clay interlayer and adsorbed water was removed or made isotopically non-exchangeable, and that non-equilibrium isotopic fractionation occurring at different temperatures during heating might be responsible for some of the differences we observed.”

6. P14 L5: Freezing of the wetted soils before loading in the CWE may reduce vapour loss during evaporation

Response: We did not include any recommendations on freezing the samples before water extraction. However, we agree with the reviewer and most labs indicated that they have frozen the samples before the actual water extraction to prevent evaporative water losses.

7. P17 L22-23: This sentence seems adrift here, but it is a valid point that should be expanded upon, possibly in the introduction/background. It is a valid question to ask whether it is actually relevant to extract all water from a soil sample - it will depend on the study context.

Response: We will discuss this aspect in more detail and shift it to the discussion section.

8. P17 L29: Not all laser instruments (LAS) were of the OA-ICOS type (Los Gatos) in the WICO study (Wassenaar et al 2018) - several were CRDS instruments (Picarro). This section should also be modified with respect to organic interferences in LAS, the effects can be dramatically different between Los Gatos and Picarro instruments both in direction and magnitude. They are even different between different generations of Picarros (see e.g. Munksgaard et al. Rapid Commun. Mass Spectrom. 2014, 28, 2151–2161)

Response: We will edit this paragraph following the reviewer’s suggestions.

9. P18 L28: Interferences can also be overcome by in-line high temperature oxidation prior to LAS measurement, although this will likely contribute small amounts of H_2O which may or may not be significant compared to the overall extraction amount.

Response: We will add this point to the discussion section: “Martín-Gómez et al. (2015) introduced an on-line oxidation method for organic compounds for samples measured via isotope-ratio infrared spectroscopy. The authors showed that this method was able to effectively remove methanol interference, but was not efficient for high concentrations of ethanol.”

10. P20 L11: Does this mean that in effect each soil would have to be investigated (i.e. a standard addition technique) unless a series of samples have very similar contents and type of organics and clay? - a very tedious process.

Response: So far, we do not see any other possibility.

References

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