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.

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Reply to Reviewer 2

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

This work provides a good initiative towards the standardization of a procedure that can be carried out in multiple ways. The inclusion of laboratories worldwide depicts this need as well as the large variety of extraction systems developed to carry out the CWE. The idea of involving multiple laboratories under the same approach is well done and supported with a good protocol (despite the possible sources of errors described by the authors). However, even if the authors do not mention a specific method or guidelines at the end of the paper; the information provided can lead to the best practice. On the Methods (page 5, lines: 27-32) and Discussion (page 14, lines: 15-22) sections, the authors mention the “performance test” carried out by the laboratories. The analysis of this data needs more attention on the Results section and it can be integrated with a Zscore graph as the one showed by Orlowski et al (2016). This type of plot will help to see the proportional laboratory efficacy to reach the labeled water. In addition, it will be important to add information about the performance test carried out by the laboratories, because the paper only mentions the data of two laboratories and leave on the dark the data from the other 14. The laboratory capacity to successfully extract soil water is essential for most of the projects relying on that information (the reason why the authors defined the experiment). This work shows that despite having identical soil and water samples, as well as the protocol extraction (“pre-defined extraction method”), no one of the laboratories was able to reach the water signature. The proposed rehydration process could be affected by small differences among the soil samples sent to each laboratory (nonhomogeneous composition between subsamples of the same soil). This brings the question if different subsamples of soil were analyzed to test the homogeneity among samples as the authors did with the water? In addition, did any of the laboratories send a sample of rehydrated soil for its physical and chemical analysis? Because this can help the authors to support their assumption (Discussion section, page 17; 13-15) Despite the authors sentence (Discussion section, page 20, lines: 1-2): “We found no clear tendency for which approach should be applied, thus at present, and much to our dismay, we cannot define any standard protocol for CWE”; the information contained in this paper can give important clues about the feasibility of applying one specific method. If the authors apply the Zscore graph (Orlowski et al, 2016) mentioned previously (second paragraph); they can determine which methods lead towards a more accurate extraction among all the setups evaluated considering the pre-defined protocol (analysis 1) and considering the laboratory protocol (analysis 2). In this way, the authors can provide as “take-home messages” the laboratory practices that lead towards better results. If the authors change the notation from 20. The amount of sample material used per laboratory is not reflected in table 1 and this information can help to understand the differences.

Response: We thank referee #2 for the very valuable and positive comments, which helped us to improve our manuscript.

Figures 3 and 4 provide mean differences to the reference DI water allowing assumptions on how good or bad individual labs performed with respect to the “reference”. Simply put, a Z-score is a numerical measure of a value's relationship to the mean in a group of values. If a Z-score is 0, it represents the score as identical to the mean score. Figure 3 and 4 provide this information, not in

dual isotope space but individually for each isotope. The figures further include the SD of the lab's extraction results. It is this information that the paper intends to convey. We therefore would prefer, and we hope our argument above helps to demonstrate this, not to provide additional Z-score plots for the soil water extraction results. They would be redundant in some ways and would not convey the precise message we intend with Figures 3 and 4 in their current form.
With respect to the "performance test" results, we did not include these results in our manuscript since not every lab provided the full set of data. We therefore picked some examples as the data allowed. Again, a great suggestion but our data limited us in this regard.

We can rule out any inhomogeneity of the bulk soil sample's soil physico-chemical properties. The LUFA Speyer provides "standard soils" exactly for conducting lab studies (https://www.lufa-speyer.de/index.php/dienstleistungen/standardboeden/8-dienstleistungen/artikel/57-standard-soils). The LUFA already dried, homogenized and sieved (with a 2 mm screen) the bulk soil sample according to "Good Laboratory Practice". However, we repeated these steps before taking subsamples for the individual labs. Since every lab rehydrated the soil samples with the same deionized water (that we sent to them) and soils were only used once per extraction, we assume that potential changes in the soil properties due to the water additions are the same across labs. We did not see the necessity in determining the soil properties for a second time after water extraction, since samples were not extracted multiple times. Soil properties were determined according to German DIN-ISO norms on dried soil samples (e.g., DIN-ISO 11465, DIN-ISO 10390). We therefore did not see the need in analysing soil properties on a rehydrated soil sample.

With respect to the "take-home-message", we will further add the following: "Orlowski et al. (2018) recently explored the effect of CWE for tracing plant source water. The authors tested the ability to match plant water to its putative soil water source(s) by using different CWE conditions (30–240 min, 80–200 °C, 0.1 Pa) for a clayey loam (same as in this study) and a sand. They showed that with higher extraction temperatures and longer extraction times, gradually more enriched soil water was extracted, which surprisingly reflected the plants' source water…For most past studies, possible fractionation effects associated with CWE remain unknown and the applied extraction parameters or cryogenic system specifications are often not indicated. Orlowski et al. (2018) recently stated that observed isotopic fractionation effects potentially lead to errors when CWE isotope data is used for plant water source calculation. This miscalculation in plant's water source could be quite large and could lead to misinterpretations of the role different plant species play in hydrologic processes at the ecosystem or larger scales. Millar et al. (2018) used the most common water extraction methods (centrifugation, microwave extraction, direct vapor equilibration, high-pressure mechanical squeezing, and two different CWE systems) for their intercomparison study on spring wheat (Triticum aestivum L.). The authors showed that all methods yielded markedly different isotopic signatures. The various methods also produced differing concentrations of co-extracted organic compounds. Again, CWE was outperformed by other extraction methods...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."

We will include the amount of sample material used per laboratory in Table 1. We will further include the following in the 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."

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Reply to Reviewer 3

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

General comments

This manuscript reports the results of an intercomparison exercise that aimed at testing the consistency of cryogenic water extractions for the analysis of stable isotope of hydrogen and oxygen in soil water among worldwide-distributed laboratories. In the last few years, the ecohydrological and soil science international communities have shown a strong and increasing interest in better understanding the functional interrelationships between soil and vegetation based on the use of stable water isotope data. The cryogenic water extraction technique has so become a golden standard for sampling water from the unsaturated zone. Very recent studies, often conducted by the first author of this manuscript and colleagues, already showed some potentials and limitations of this technique, and provided helpful information for users. However, a worldwide interlaboratory comparison among several cryogenic extraction facilities was still missing, thus this work it is certainly welcome. Indeed, I believe that this manuscript is timely and of great interest for the readers of this journal. The manuscript is very well written, logically structured, nicely illustrated, and the conceptual steps can be followed very well. The working hypothesis and the specific objectives are well posed, following a substantial introduction, the statistical analysis is correct, and the results and interpretation are well supported by the data. The methodological approach leading to the comparison exercise was solidly defined and clearly presented. As noted by the Authors themselves, the large differences in performance found among the labs included in the exercise are somehow worrisome and pose questions on the possible adoption of cryogenic water extraction as a standard method for soil water sampling. However, these results are very relevant to the scientific community because implicitly suggest cautions in comparing isotope soil water data extracted by different facilities, and indicate that much technical work is still needed to test possible further controls on these differences and develop new techniques able to return more consistent results.

We thank Daniele Penna for taking the time to review our manuscript and providing this very positive feedback.

Specific comments

1. P4 L11. Here, and throughout the rest of the manuscript (e.g., P7 L16; P9 L12-14; P11 L15. . .), it is not immediately clear what "isotope results" are, and I suggest to replace this term with "values" or "data".

Response: We will change it to "data" or "values" or "composition" as suggested.

2. P5 L14. The authors reported that the soil was homogenized before shipping. However, as noted by the other reviewers, I wonder if possible heterogeneities in the analysed soil samples (especially for small volumes) could have been present and could have affected the results.

Response: For our intercomparison, we used "standard lab soils" from the LUFA Speyer (https://www.lufa-speyer.de/index.php/dienstleistungen/standardboeden/8-dienstleistungen/artikel/57-standard-soils). In guidelines of the German JKI (Julius-Kuehn-Institute) and other relating guidelines (OECD), soils with certain characteristics are recommended for such studies. The LUFA lab already provided dried, homogenized and sieved soils (with a 2 mm screen) according to “Good Laboratory Practice”. However, we repeated the homogenization and oven-drying process to ensure that all subsamples that we shipped to the respective labs were equal.

3. P5 L27. I find the definition of “water-water” cryogenic extraction a bit confusing. I suggest to use, throughout the manuscript, simply the terms “water extraction” vs. “soil water extraction”, or something similar.

Response: We will follow the reviewer’s suggestion.

4. P7 L1-2. Although intuitive, I suggest to add a short explanation about the choice of applying different extraction times for the silty sand and the clayey loam soils.

Response: We will add the following to the revised version of the manuscript: ”Since different extraction times and temperatures were applied in past studies, we decided that participating laboratories should follow two different extraction approaches...” and further “For comparison, in past studies extraction times from 2.5 min (Koeniger et al., 2011) over 30 min (West et al., 2006) to 7 h (Araguás-Araguás et al., 1995) for sandy soils and from 30 min (Goebel & Lascano, 2012) over 40 min (West et al., 2006) to 8 h (Araguás-Araguás et al., 1995) for clayey soils were reported.”

5. P8 L28, and P12 L6-20, and Figs. 5-6. It seems to me that these are not “true evaporation lines” describing the progressive isotopic enrichment of an individual source water (see Benettin et al., 2018 who reported the often misused term and concept of evaporation line). This might not be a critical point in the interpretation of the results and the overall meaning of the research. However, for the sake of accuracy, I suggest to check this and in case change the terminology (eg, simply calling them regression lines) and slightly re-interpret the results reported at P12 L6-20. Moreover, it’s not very clear to me why in the left panels of Figs. 5 and 6 (8% WC) one regression line (for 8% samples) is reported in addition to the GMWL whereas in right panels of Figs. 5 and 6 (20% WC) two regression lines are shown (both for 8% and 20% WC). Please, fix this or explain.

Response: We will change the term to “regression lines”. We will further add the following to the respective section on P12: “Benettin et al. (2018) recently revised the widely used concept of evaporation lines. The authors question that the trend line passing through fractionated soil water samples correctly identifies their source water and emphasis that trend lines through evaporated samples can differ widely from true evaporation lines.”
For clarity, we deleted regression lines for the 8% WC versions from the right panels of Figures 5 and 6.

6. P9 L23-24. Do the Authors have any idea about the reason for recovery rates higher than 100%? Could this somehow affect the results? Perhaps a sentence could be added here (trying to avoid the risk of speculation).

Response: We already included a potential explanation for this phenomenon in the discussion section of our previous manuscript version: “This could be attributed either to leaky vacuum systems (which might allow atmospheric water vapor to enter the system) or to a remoistening of the oven-dried soil samples before water extraction."

7. P11 L27. In addition to the statistical results, I wonder whether it might be appropriate to show OA-ICOS and IRMS data as boxplots to graphically stress the difference between values returned by the two techniques.

Response: Figures 3 and 4 show the mean of the three replicates with respect to the reference DI water and y-error bars stand for the isotopic variation of the replicates. Since our sample size (n=3) is relatively small, we decided to display only the data's central tendency by using traditional mean-and-error scatter plots. The decision follows recommendations by Nature methods (2014) and we hope that the reviewer will concur with this assessment (a great suggestion though, if we had a larger n).

8. P17 L23. The reference of "Orlowsky et al., 2018" is missing from the reference list.

Response: We will add the missing reference.

9. Fig. 1. I suggest to increase the size of the axis labels.

Response: We will follow the reviewer's suggestion.

10. Fig. 3 and Fig. 4. In the caption: was the mean computed among the three replicates? If so, I suggest to specify this for the sake of clarity.

Response: We will edit the sentence as follows: "Symbols represent the mean of the three replicates and y-error bars stand for the isotopic variation of the replicates."

11. Fig. 5 and Fig. 6. I suggest to add in the caption that the legend includes explanation for the symbols used for the 16 labs and the two extraction approaches.

Response: We will add the following: "…for OA-ICOS and IRMS data (upper and lower panels, respectively) from the 16 participating labs (different colors represent different labs) and both extraction methods (lab-procedure: I and pre-defined: II). For reference, plots include the Global Meteoric Water Line (GMWL, solid red line) and soil water regression lines for 8% and 20% WC (solid green and orange lines, respectively)."

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Inter-laboratory comparison of cryogenic water extraction systems for stable isotope analysis of soil water

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Abstract

For more than two decades, research groups in hydrology, ecology, soil science and biogeochemistry have performed cryogenic water extractions for the analysis of δ^2H and δ^{18}O of soil water. Recent studies have shown that extraction conditions (time, temperature, and vacuum) along with physicochemical soil properties may affect extracted soil water isotope ~~results~~composition. Here we present results from the first worldwide round robin laboratory intercomparison. We test the null hypothesis that with identical soils, standards, extraction protocols and isotope analyses, cryogenic extractions across all laboratories are identical. Two ‘standard soils’ with different physicochemical characteristics along with deionized reference water of known isotopic composition, were shipped to 16 participating laboratories. Participants oven-dried and rewetted the soils to 8% and 20% gravimetric water content, using the deionized reference water. One batch of soil samples was extracted via pre-defined extraction conditions (time, temperature, and vacuum) identical to all laboratories; the second batch was extracted via conditions considered routine in the respective laboratory. All extracted water samples were analyzed for δ^{18}O and δ^2H by the lead laboratory (Global Institute for Water Security, GIWS, Saskatoon, CA) using both a laser and an isotope ratio mass spectrometer (OA-ICOS and IRMS, respectively). We rejected the null hypothesis. Our results showed large differences in retrieved isotopic signatures among participating laboratories linked to soil type and soil water content with mean differences to the reference water ranging from +18.1‰ to –108.4‰ for δ^2H and +11.8‰ to –14.9‰ for δ^{18}O across all laboratories. In addition, differences were observed between OA-ICOS and IRMS isotope data. These were related to spectral interferences during OA-ICOS analysis that are especially problematic for the clayey loam soils used. While the types of cryogenic extraction lab construction varied from manifold systems to single chambers,

no clear trends between system construction, applied extraction conditions, and extraction results were found. Rather, observed differences in the ~~between~~ isotope ~~results~~ data were influenced by interactions between multiple factors (soil type and properties, soil water content, system setup, extraction efficiency, extraction system leaks, and each lab's internal accuracy). Our results question the usefulness of cryogenic extraction as a standard for water extraction since results are not comparable across laboratories. This suggests that defining any sort of standard extraction procedure applicable across laboratories is challenging. Laboratories might have to establish calibration functions for their specific extraction system for each natural soil type, individually.

1 Introduction

The interpretation of the stable isotope signatures of water (δ^2H and δ^{18}O) from soils in many research disciplines relies on accurate, high-precision measurements (Wassenaar et al., 2012). To extract water from soils for isotopic analysis, cryogenic water extraction (CWE) is the most widely used laboratory-based removal technique (Araguás-Araguás et al., 1995; Orlowski et al., 2016a). The ability to obtain measurable amounts of water from small sample sizes (i.e. < 10 g) makes this method attractive. However, CWE is also accompanied by high capital and operating costs. Despite its widespread use, recent work has identified several extraction artifacts leading to uncertain isotopic signature identification (Gaj et al., 2017a; Orlowski et al., 2016b). Studies have shown that extraction conditions (i.e., extraction time, temperature, and vacuum) need to be adapted specifically to the soil used (Araguás-Araguás et al., 1995; Gaj et al., 2017a; Meißner et al., 2014; Orlowski et al., 2016a)~~(Araguás-Araguás et al., 1995; Gaj et al., 2017a; Meißner et al., 2014; Orlowski et al., 2016a)~~. Notwithstanding, isotope effects triggered by physicochemical soil properties (e.g., clay minerals, soil organic carbon content, and water content) can occur (Araguás-Araguás et al., 1995; Gaj et al., 2017a; Meißner et al., 2014; Oerter et al., 2014; Orlowski et al., 2013)~~(Araguás-Araguás et al., 1995; Gaj et al., 2017a; Meißner et al., 2014; Oerter et al., 2014; Orlowski et al., 2013)~~. However, the ecohydrology and soil science communities currently lack clear recommendations for standardized water extraction conditions from soils. Although there seems to be an agreement on the need to control the extraction yield of cryogenic extraction facilities (recovery rate in percentage of previously added water), there exists a large variability in the applied extraction conditions between laboratories. Moreover, extraction systems vary in terms of heating elements, size of extraction containers, or throughput, in addition to the aforementioned extraction conditions (Goebel and Lascano, 2012; Koeniger et al., 2011; Orlowski et al., 2013). Thus, no standard system setup or methodology exists.

Despite the work to date and the extensive application of stable water isotope analysis, no formal interlaboratory comparison between different cryogenic systems has been published. Here we present the first worldwide interlaboratory comparison between 16 different cryogenic extraction facilities. CWE procedures were conducted with two standard soils with different physicochemical characteristics (silty sand and clayey loam), spiked with a known isotopic label at different gravimetric water contents (WC of 8% and 20%). The null hypothesis guiding this work was that all laboratories would yield the same results independent of soil type and water content. In addition, we addressed the following research questions:

1. How does the cryogenic system configuration affect resulting soil water isotopic composition?
2. How do soil type and soil water content affect the isotope ~~results~~data?
3. How do results differ when extracted soil water stable isotopic compositions are measured via off-axis integrated cavity output spectroscopy (OA-ICOS) vs. isotope ratio mass spectrometry (IRMS)?
4. What do we learn from this exercise for standardization of cryogenic extraction facilities?

2 Methods

2.1 Experimental design

Table 1 provides a description of the respective extraction systems that participated in the intercomparison. In total, 16 independent laboratories from seven countries took part in the trial.

[Table 1 near here]

Before the commencement of the round robin test, participants were asked to fill out a questionnaire (see Appendix 1) to characterize their cryogenic extraction system in terms of numbers of extraction slots or amount of sample material usually introduced into the system (size of extraction unit). Two standard soils with different physicochemical properties (clayey loam and silty sand) from the German State Research Institute for Agriculture (LUFA Speyer, 2015) (Table 2) were used for the interlaboratory comparison.

[Table 2 near here]

We chose a silty sand from which we expected water extractions to be relatively easy for each laboratory without cation ion exchange problems, and a clayey loam soil,—which is known to be challenging for CWE extraction systems. Clayey soils can be difficult due to interactions with the clay fraction and different types of clay minerals—the so-called adsorbed cation effect (Oerter et al., 2014). Clay soils also present challenges with regard to the tightness of water bound to mineral surfaces which causes an additional isotope effect (Ingraham and Shadel, 1992; Oerter et al., 2014; Walker et al., 1994).

Soil samples were sieved to a grain size <2 mm. Soils were pre-dried at 105°C for 48 h, homogenized, and shipped in tightly sealed glass bottles to the 16 independent laboratories along with deionized (DI) reference water of known isotopic composition (measured on both an IWA-45EP Analyzer (OA-ICOS, Los Gatos Research Inc., Mountain View, US): δ^{2}H: −59.8±0.2‰ and δ^{18}O: −8.5±0.1‰, n=6; and via Delta V™ Advantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA, US): δ^{2}H: −60.5±0.2‰ and δ^{18}O: −8.7±0.1‰, n=6). All bottles containing either soils or DI water were filled, capped tightly, and wrapped with Parafilm® to prevent water loss. We decided not to ship ready-to-use rehydrated soils to avoid evaporation fractionation effects and to give participants the opportunity to adjust e.g. samples sizes to the specific requirements of their extraction system. Water loss and evaporative enrichment from the shipped DI water was checked by isotopic comparison of shipped and non-shipped DI water (1. shipment test: Giessen–Freiburg (Germany)–Saskatoon (Canada) and 2. shipment test: Giessen (Germany)–Saskatoon (Canada) vs. non-shipped water samples). After this simple experiment, isotope fractionation effects due to shipment were excluded.

As a reliability test, each participant in the intercomparison performed water~~–water~~ cryogenic extractions (defined here as simply extracting pure water, i.e. without any soil material present) using their extraction facility. This was done in order to determine the capability of the respective extraction apparatus to recapture water of known isotopic composition. After showing the operational reliability, CWEs with the rehydrated soil samples were performed following a pre-defined protocol.

2.2 Sample preparation protocol

Before starting the rewetting of the pre-dried soil samples with the DI water, participants oven-dried (at 105°C for 48 h) the provided soils again to remove any potential water that could be present (e.g., remoistening of the soil samples during shipment). Afterwards, soils were placed in a desiccator for cooling and to prevent remoistening of the dried soil samples with ambient water vapor (Orlowski et al., 2016b; Van De Velde and Bowen, 2013). For rehydration, two different amounts of reference DI water were added to the respective soil types (to create 8% and 20% gravimetric WC). Exposure of the dried soil samples to ambient conditions was kept as short as possible. Participants adjusted the amount of respective soil material and water for rewetting the samples according to the specific requirements of their extraction system e.g., size of extraction containers. Sample preparation was performed separately for OA-ICOS and IRMS analysis but in the identical way as specified below:

1. Soil and DI water were added alternately~~in an alternating fashion~~. A quarter of soil material (clayey loam/silty sand) and a quarter of DI water were alternatively added to the pre-weighed extraction tube to facilitate soil-water-homogenization.
2. This rewetting procedure was completed by adding a quarter of soil material to the extraction tube to avoid supernatant water and to obtain the best possible mixing.
3. Samples were weighed again.
4. Finally, an inert cover (Fackelmann Inc, Hersbruck, DE) was placed on top of the soil sample to avoid the spread of sample material throughout the respective cryogenic extraction line. The inert material was proven to not cause isotope effects during soil water extraction (Orlowski et al., 2013).
5. Extraction tubes were plugged and sealed with Parafilm® to ensure an air-tight system.
6. Rehydrated soils in their respective extraction containers were placed in vertical position in a refrigerator (5°C for 72 h), which further allowed the liquid and solid phase to equilibrate.

2.3 Cryogenic extraction approaches

Since different extraction times and temperatures were applied in past studies, we decided that participating laboratories should ~~Each laboratory was instructed to~~ follow two different extraction approaches: (I) For the first subset of rehydrated soil samples, participants applied the CWE procedure considered routine in their laboratory for the specific soil type and soil water content. (II) With the second subset, CWE under pre-defined conditions for all labs was performed: For silty sand, a 45 min extraction time was used while 240 min was applied to clayey loam samples, both at an extraction temperature of 100°C and a vacuum of 0.3 Pa. These pre-defined extraction parameters were identical for all participating laboratories. For comparison, in past studies extraction times from 2.5 min (Koeniger et al., 2011) over 30 min (West et al., 2006) to 7 h (Araguás-Araguás et al., 1995) for sandy soils and from 30 min (Goebel and Lascano, 2012) over 40 min (West et al., 2006) to 8 h (Araguás-Araguás et al., 1995) for clayey soils were reported.

Three replicates per soil type and soil water content resulting in 24 samples per extraction procedure (pre-defined and laboratory specific) and isotope analysis method (OA-ICOS and IRMS) were processed (n=48 in total). Pre- and post-oven-dried (105°C for 24 h) soil sample weights were used to determine water recovery rates. All extracted water samples were transferred to 2 mL amber glass vials capped with solid lids (Th. Geyer Inc., Renningen, DE), tightly sealed with Parafilm®, labeled, and shipped to the GIWS for isotope analysis. If the amount of extracted water was not sufficient to entirely fill the 2 mL vial, inserts (0.2 mL) were used (Th. Geyer Inc., Renningen, DE) to minimize sample vial headspace, following standard procedures as outlined by the IAEA (2014).

2.4 Isotope analyses

For cross-checking isotope data~~results~~ and ruling out potential lab analytical differences, the isotopic composition of the extracted water samples was analyzed via both OA-ICOS and IRMS. OA-ICOS samples were analyzed on an IWA-45EP Analyzer (Los Gatos Research Inc., Mountain View, US). The accuracy of OA-ICOS analyses was ±0.5‰ for δ^2H and ±0.1‰ for δ^{18}O. IRMS samples were analyzed on a Delta V™ Advantage mass spectrometer (Thermo Fisher Scientific, Waltham, MA, US) and an H/Device peripheral using a Cr-reduction method for δ^2H analysis (Morrison et al., 2001). For δ^{18}O analysis, a GasBench II peripheral was utilized. Using mass spectrometry, a conversion from the water into a light gas suitable for mass spectrometry (H_2, CO_2, CO, O_2) is necessary. This conversion step often turns out to limit the achievable precision of IRMS (Brand et al., 2009). In our case, IRMS results are accurate to ±1‰ for δ^2H and to ±0.2‰ for $\delta^{18}O$, respectively. All isotope ratios are reported in per mil (‰) relative to Vienna Standard Mean Ocean Water (VSMOW) (δ^2H or $\delta^{18}O=(R_{sample}/R_{standard}-1)\times1000‰$), where R is the isotope ratio of the sample and the known reference (i.e. VSMOW)) (Craig, 1961). In-house standards, calibrated against VSMOW2 and SLAP2, were run as samples to allow the results to be reported against VSMOW (Nelson, 2000).

OA-ICOS isotope data of soil water extracts were checked but not corrected for spectral interferences (caused by potentially co-extracted organics such as methanol or ethanol) using the Spectral Contamination Identifier post-processing software (LWIA-SCI, Los Gatos Research Inc.) when measured via OA-ICOS. This software compares recorded spectra from unknown samples with those from known non-contaminated samples (standards) to produce a metric of contamination from either narrow-band (e.g., methanol (MeOH)) or broad-band (e.g., ethanol (EtOH)) absorbers which indicates the likelihood or degree of spectral interference (Schultz et al., 2011). IRMS results are generally not affected by organic contaminants.

2.5 Statistical evaluation

We used R for statistical analyses (R version 3.3.2; R Core Team, 2014). For quantifying laboratory variances, differences between pre-defined and laboratory specific extraction procedures, effects of soil type and WC, differences between OA-ICOS and IRMS, all data were tested for normality using the Shapiro-Wilk test. Homoscedasticity was tested using either the Levene’s test for normally distributed data or the Fligner-Killeen test for non-normally distributed data. Cook’s distance was determined in order to identify outliers (D>1). Depending on the type of data (normally distributed and homoscedastic), either Kruskal-Wallis rank sum tests or Analyses of Variances (ANOVAs) were applied and posthoc tests (e.g., Nemenyi-tests) were run to determine which groups were significantly different ($p\leq0.05$). P-value adjustments via the FDR-method (false discovery rate) were applied to reduce the family-wise-error rate (Zieffler et al., 2012).

For graphical comparisons, a target standard deviation (TSD) for acceptable performance was set to ±2‰ for δ^2H and ±0.2‰ for $\delta^{18}O$ similar to Orlowski et al. (2016b), which is considered reasonable for hydrologic studies (Wassenaar et al., 2012). The TSD does not account for errors associated with the extraction method itself, weighing errors, and volumetric water additions to the sample, or any standard deviations (1SDs) related to the isotope analysis. Statistically significant ($p \leq 0.05$) linear regressions were added to dual isotope plots as references ~~(evaporation water lines)~~ as well as the Global Meteoric Water Line (GMWL: $\delta^2H = 8.2 \times \delta^{18}O + 11.3‰$, as defined by Rozanski et al. (1993)).

3 Results

3.1 Cryogenic extraction systems and water extraction efficiencies

Cryogenic extraction systems varied greatly from lab to lab: from manifold, high-throughput devices (as described by Orlowski et al. (2013)) to small, single chamber systems (as in Koeniger et al. (2011) and West et al. (2006)) (for details see Table 1). The systems showed differences in terms of the extraction containers (form, size, volume, and material), the heating module and its application temperature (heating tapes or lamps, water baths or hot plates), the type of fittings and connections (glass, stainless steel), as well as in the vacuum producing units (Table 1). 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.

To determine the degree of extraction efficiency for each lab's samples, water recovery rates were calculated for those labs that provided the complete set of soil weight data (in % of previously added water). When comparing water recovery rates against δ^2H and $\delta^{18}O$ values~~isotope results~~, the clayey soil showed no clear trend (Fig. 1). Even if water recovery rates were higher than 98% (following the definition of (Araguás-Araguás et al., 1995)~~Araguás-Araguás et al. (1995)~~), extracted isotope ~~results~~ values differed from the reference DI water (Fig. 1). For example, at 8% soil water content (WC), recovery rates of above 98% were achieved, but isotope ~~results~~ values were depleted in comparison to the reference DI water (Fig. 1, left panels).

[Figure 1 near here]

For the silty sand, recovery rates were generally higher in comparison to the clayey soil. Only a few samples showed extraction efficiencies lower than 98% (Fig. 1, right panels).

Surprisingly, we observed some recovery rates higher than 100%. This was especially an issue for soils at 8% WC (Fig. 1).

Correlation analysis was performed in order to relate extraction parameters (i.e., time, temperature or vacuum) to OA-ICOS and IRMS isotope ~~results~~data.

[Figure 2 near here]

We found no significant correlations between the extraction parameters and the respective isotope ~~results~~data, exemplarily shown for δ^2H results (Fig. 2) (e.g., R^2=0.0 for δ^2H vs. duration or temperature).

3.2 Laboratory performance with respect to water content and soil type

Figures 3 and 4 show the mean differences between the extracted samples via the lab procedure's extraction approach (I) and the pre-defined extraction approach (II) and the reference DI water δ^2H and δ^{18}O values, respectively.

For the 8% WC tests, mean differences for the clayey loam ranged from +13.1 to −32.8‰ for δ^2H. For the individual lab procedure's extraction approach (I) at 8% WC for the clayey loam, two laboratories (lab 3 and 8) were able to get back to the reference δ^2H value based on no statistically significant differences ($p>0.05$) (Fig. 3, upper left plot). For the pre-defined extraction approach (II) at 8% WC, two other labs recovered the δ^2H value from the clayey loam (lab 9 and 15).

For soil samples with 20% WC, variation among laboratories was smaller but only one laboratory (lab 9) recovered the reference DI water δ^2H value applying the pre-defined extraction approach for the clayey loam. Mean differences between the clayey loam extracts and the reference DI water ranged from +2.8 to −19.5‰ (Fig. 3, upper right plot).

[Figure 3 near here]

Mean differences between the silty sand water extraction and the reference δ^2H signature were in a smaller range of ±18‰ than clayey loam extracts from the same treatment (8% WC).

For the individual lab procedure's extraction approach (I) at 8% WC, five laboratories recovered the added label from the silty sand (Fig. 3, lower left plot) with no statistical differences between the reference DI water ($p>0.05$) (labs 6, 8, 9, 13, and 15), whereas for the pre-defined extraction approach (II) at 8% WC, three labs got back to the added δ^{2}H value (labs 9, 12, and 15).

For silty sand at 20% WC, most laboratories' results even fell close to the range of the TSD of ±2‰. Mean differences to the reference DI water δ^{2}H signature ranged from +8.5 to −15.1‰ (Fig. 3, lower right plot). However, extraction approach I was statistically not successful in recovering the added label ($p<0.05$), but five laboratories (6, 9, 10, 14, and 15) showed no significant differences to the reference DI water when applying extraction approach II to the silty sand at 20% WC.

Laboratories performed better for δ^{18}O signature recovery, especially with extraction approach I. For both clayey loam WC treatments, labs 13 and 15 were the most successful. Again, mean differences to the reference DI water were larger for the 8% WC than for the 20% WC (Fig. 4, upper plots). However, for the clayey loam at 20% WC with the pre-defined approach (II) only lab 13 and 14 did not show statistically significant differences to the added δ^{18}O signature (Figure 4, upper right plot) ($p>0.05$).

[Figure 4 near here]

For the silty sand, most laboratories were able to get back the known value with no statistically significant differences to the reference δ^{18}O value (Fig. 4, lower plots). For both WC treatments of the silty sand, extraction approach II seemed to work better in recovering the added label.

Across both soil types, WC treatments, and extraction approaches, lab 13 was the most successful in recovering the reference δ^{18}O value, whereas for δ^{2}H recovery lab 9 gained back the added label in most of the cases.

In general, δ^{2}H and δ^{18}O values ~~isotope results~~ were neither comparable between laboratories nor between one laboratory at different soil types or WCs, meaning that a specific laboratory, for example, successfully recovered the added DI water value for silty sand but was not able to gain back the known label for clayey loam. Moreover, recovery results differed between both isotopes. For example, lab 13 was the most successful for δ^{18}O but not for δ^{2}H signature recovery. In terms of lab internal reproducibility, some labs showed small standard deviations for the replicates of the same soil type at a given WC (Fig. 3 and 4); even so, resulting isotope values~~results~~ differed statistically significant from the introduced reference DI water.

3.3 Differences between OA-ICOS- and IRMS-based measurements

Figures 5 (clayey loam) and 6 (silty sand) illustrate data variability for each laboratory and WC with respect to the labeled reference DI water added to each soil type in dual isotope space. Significant differences were observed between OA-ICOS and IRMS isotope data sets ($p\leq0.05$). The clay soil isotope data at 8% WC showed the greatest differences between OA-ICOS and IRMS measurements (mean differences of 1.3 and 1.2 for δ^{2}H and δ^{18}O, respectively). Smallest differences between isotope analyzers were observed between both WC treatments of the silty sand (Fig. 6). The data sets with the lowest SD for both isotopes across labs and extraction approaches were the silty sand samples at 20% WC measured via OA-ICOS and IRMS (SD of ±3.1 for δ^{2}H measured via OA-ICOS and ±4.2 for IRMS, respectively). However, those data sets still did not reach the TSD of ±2‰ for δ^{2}H and ±0.2‰ for δ^{18}O.

[Figure 5 near here]

For comparison, apart from ~~evaporation~~ soil water regression lines, the GMWL is also given in each subplot. Interestingly, isotope data across laboratories plot on slopes lower than the GMWL. For both soil types, ~~evaporation~~ regression lines of the IRMS measurements showed better correlations (for the silty sand $R^{2}=0.8$ and 0.9 for 8% and 20% WC, respectively) than those of OA-ICOS measurements ($R^{2}=0.7$ for 8% and 20% WC) (Fig. 6). Silty sand's soil ~~evaporation~~ water regression lines showed greater slopes (5.4–7.2 across both WCs and isotope analysis) than clayey loam's soil ~~evaporation~~ water regression lines (2.8–5.2 across both WCs and isotope analysis) (Figures 5 and 6). The clayey loam ~~evaporation~~ regression lines for the higher WC also showed greater slopes than those of the lower WC (Fig. 5). Isotopic fractionation due to evaporation leads to a stronger kinetic effect for ^{18}O compared to ^{2}H, resulting in evaporative enrichment of the water along an evaporation water line ~~(e.g., soil evaporation water line)~~ with a lower slope relative to the original water (Gonfiantini, 1986)~~ in our case, the reference DI water~~. Benettin et al. (2018) recently revised the widely used concept of evaporation lines. The authors question that the trend line passing through fractionated soil water samples correctly identifies their source water and emphasis that trend lines through evaporated samples can differ widely from true evaporation lines.

For the clay soil type, the IRMS data sets (8% and 20% WC) plot closer to the GMWL and the analyzed values showed a smaller SD in comparison to the OA-ICOS assays (SD of ±8.4 for the OA-ICOS δ^2H data vs. ±7.5 for the OA-ICOS data at 8% WC) (Fig. 6).

[Figure 6 near here]

In general, the spread of the isotope data decreased from 8% to 20% WC and from OA-ICOS to IRMS measurement results (Figures 5 and 6). The OA-ICOS isotope analyses showed more outliers than those of IRMS. Moreover, fewer outliers were found among the silty sand data when compared to that of the clayey loam soil. Overall, IRMS results for all soil types and WCs were slightly more depleted than those of OA-ICOS. However, differences were not significant ($p>0.05$). In general, most of the water extracts were depleted in comparison to the reference DI water, which is especially true for δ^2H.

Examination of the differences between OA-ICOS and IRMS data, prompted to test the OA-ICOS data for spectral interferences. Figure 7 shows that for the clayey loam soil, differences between OA-ICOS and IRMS data might be due to co-extracted alcoholic compounds, which caused erroneous OA-ICOS data.

[Figure 7 near here]

Few samples among the 8% WC versions of clay water extracts showed issues with both broad-band and narrow-band absorbers. This contamination by both methanol and ethanol explained the outliers found at 8% WC in the clayey loam data (Fig. 7, upper left plot). Among these data, only a small number of samples showed no contamination, which were interestingly more depleted in comparison to data flagged as affected by narrow-band absorbers. For the silty sand soil, only a few samples were contaminated and flagged as affected by narrow-band absorbers. Interestingly, outliers in the silty sand soil data set at 8% WC could not be explained by narrow- or broad-band absorbers.

4 Discussion

4.1 Why are the cryogenic extraction results different across the participating laboratories?

We rejected our null hypothesis that all laboratories would yield the same results independent of soil type and water content. We showed that cryogenic extraction results were not comparable among laboratories. We also observed differences in the ability of individual labs to recover both isotope values (δ^2H and δ^{18}O) of the added reference DI water. Some laboratories were able to get back to the reference δ^2H value but were not successful for δ^{18}O.

Each extraction system setups were different. Therefore, it was difficult to give any recommendation with regard to a high-performance and accurate extraction system that would lead to overall successful extractions. As a quality control, we checked water recovery rates, which were in some cases even higher than 100% (Fig. 1). This could be attributed either to leaky vacuum systems (which might allow atmospheric water vapor to enter the system) or to a remoistening of the oven-dried soil samples before water extraction. Remoistening of oven-dried soil samples might be a general problem of such spiking experiments. In our case, sample preparation was not performed under an inert gas flow and, unfortunately, data on temperature and relative humidity conditions under which sample preparation took place are unavailable from the respective labs. Ambient water vapor isotopic composition measurements would have also been a relevant additional information. Contamination could also occur when an extraction system is not dried or cleaned after each extraction run, leaving moisture and/or soil material behind which would affect the next sample’s results. Other measurement uncertainties during the extraction protocol could arise from weighing errors (scale calibration and precision), the accuracy of the volume of water additions to the soil samples, transfer of the samples, loss of water vapor during evacuation of the extraction system, unsteady heating temperatures, condensation of water vapor in the extraction system, and a lack of precision of analytical and laboratory equipment.

It is also possible that participating labs did not follow the pre-defined extraction procedure (II) in the exactly same ways. Even extraction results from some individual labs for the same soil type and WC showed high SDs (Fig. 3 and 4) which questions the overall repeatability of individual water extraction results. For the first, “in-house” extraction approach (I), not all laboratories indicated the precise extraction conditions (extraction temperature, time, and vacuum) that they used for the specific soil types and WCs.

As an additional performance test, laboratories were asked to perform~~-~~ simple water~~-to-water~~ extractions to show their ability to recover water of known isotopic composition prior to soil-based tests. For example, some laboratories, like lab 2, showed a high accuracy for these water~~-to-water~~ extractions of ±0.4‰ for δ^2H and ±0.1‰ for δ^{18}O (n=119) as well as lab 16. They performed extraction tests with tap water, which resulted in no significant differences between the initial, untreated (−56.7‰ ± 0.4 for δ^2H and −9.3‰ ± 0.1 for δ^{18}O) and extracted tap water (−57.5‰ ± 0.6 for δ^2H and −9.4‰ ± 0.1 for δ^{18}O). These examples show that these labs among others were able to reach the TSD with simple ~~water-to-~~water extractions, but with soils, they were unsuccessful. This indicates that differences between the reference DI water and water spiked and extracted from soils are likely caused by interactions with soil particles.

Given our findings, we now question the standard quality controls (e.g., water recovery rate calculations and ~~water-to-~~water extractions without soil material). Quality controls with spiked soil samples may be a more effective way to demonstrate lab's internal accuracy. However, such spiking experiments as performed in our study come along with other issues as recently outlined by Gaj et al. (2017b) and Sprenger et al. (2015). Gaj et al. (2017a)~~Gaj et al. (2017a)~~ applied the Rayleigh equation (using stable isotope signatures) to calculate how much water was cryogenically extracted from pure clay minerals. They found that for samples from which water has been extracted to 100% (determined gravimetrically), the Rayleigh equation showed that only 72% of water was extracted at a temperature of 105°C. When using an extraction temperature of 205°C, the Rayleigh-estimated amount of water extracted was close to 90%, but still not 100%. This result clearly shows that despite the gravimetric quality control suggesting that all water has been extracted, isotopic differences may still exist.

Overall, laboratories 9 (for δ^2H) and 13 (for δ^{18}O) were the most successful in getting back to the DI reference water over all soil types and WCs. For the lab's in-house procedure, laboratory 9 extracted both soils for 90 min at 95°C and 0.8 Pa. Their reported water extraction efficiency was 99–100%. Glass tubes were used as extraction containers and a water bath as heating element. Laboratory 13 used different extraction parameters, which also varied slightly from sample to sample: for the clayey loam at 8% WC, extractions were conducted for 75–114 min at 150–100°C and 8–13.3 Pa. For the 20% WC, they used 266 min at 100°C and 6.7–13.3 Pa as in-house extraction parameters. For the silty sand at 8% WC, their extraction time was 15 min at 100°C and 7.3–13.3 Pa. For the 20% WC, they extracted for 30 min at 100°C and 6.7–

10.7 Pa. Lab 13 further specified that their extraction times were dictated by a decline in the pressure level indicating that no more water was evaporating from the respective sample. Extraction efficiencies for lab 13 varied between 93–127 %. Glass tubes were used as extraction containers along with a sensor-regulated tube-shaped heating element. This example shows that even for the relatively successful laboratories, extraction parameters did not seem to play a major role for achieving the reference DI water isotopic signature.

4.2 How do soil type and water content affect the results?

The adsorbed and interlayer water occurring in clayey soils can complicate the interpretation of obtained isotope data. Clay-water sorption capacity is well known (Schuttlefield et al., 2007; White and Pichler, 1959). White and Pichler (1959) found early on that montmorillonite adsorbs more water than kaolinite, illite, and chlorite, while chlorites and illites have similar water-sorption properties. The amount of water absorbed/adsorbed by clay minerals ranges from 800-500% for Na-montmorillonite (Kaufhold and Dohrmann, 2008; White and Pichler, 1959) to as low as 60% of the initial dry weight for biotite (White and Pichler, 1959). The clayey loam in our study was a vermiculite-rich (43 relative %) 2:1 clay type, while the silty sand had a negligible clay-fraction (2.6%) where illite (2:1 clay type) occurred with 28 relative % (Table 2).

Since Grim and Bradley (1940), we know that the absorbed/adsorbed water is difficult to remove. Savin and Epstein (1970) as well as Van De Velde and Bowen (2013) have demonstrated that the removal of interlayer and adsorbed water on clay soils can occur when they are heated at 100 to 300°C under vacuum conditions. After clay minerals lose all their water, their structure changes. Hence, care should be taken in order to remove clay minerals' water, but keeping their structure. Otherwise, rewetting experiments as presented here in our intercomparison might not be valid.

Savin and Epstein (1970) also observed that atmospheric vapor exchanged isotopically with interlayer water (almost completely) and Aggarwal et al. (2004) showed that this can occur within hours. This demonstrated that the isotopic composition of clay interlayer and adsorbed water can reflect the isotopic composition of atmospheric water vapor at the storage location. However, once the soil has been heated under vacuum and the interlayer water removed, the remaining water showed no evidence of isotopic exchange. 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. 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. Thus, sample preparation might have played its role, when it comes to discrepancies in lab's results, especially those at low water contents. At these low water contents, the available water fraction is small and exchange with interlayer and adsorbed water would be proportionally higher. In hindsight, repeating this work with soils dried under vacuum and at higher temperatures (i.e., 300 °C following Savin and Epstein (1970)) may help to clarify and to isolate the effect of remaining water in clay minerals. However, so far, regular oven-drying of soils is standard practice (Koeniger et al., 2011) for such rewetting experiments in the literature.

We also observed water content effects on the recovered isotope data as per Meißner et al. (2014). Cryogenically extracted ~~I~~isotope ~~results~~data across labs were closer to the added reference water isotopic composition at higher WCs. However, this isotope effect cannot be considered independent from other soil property effects such as clay mineral water interactions or effects caused by cation exchange capacity (CEC). Oerter et al. (2014) demonstrated that isotope effects due to soil type are more common in soils with high cation exchange capacity (CEC) at low WCs. This can be further exacerbated by the cations present in the soil. Those soils with high ionic potential (e.g., Ca^{2+} and Mg^{2+}) can create large amounts of structured water surrounding them (hydrated radii) compared to the bulk water in the system. From an oxygen isotope perspective, O'Neil and Truesdell (1991) showed that those cations are capable of causing fractionation between bound and bulk soil water. Moreover, soils higher in potassium ions may have a greater effect on hydrogen isotopes, while sodium soils demonstrate non-fractionating effects (Oerter et al., 2014). These cation fractionation effects for montmorillic soils, in particular, can result in a depletion of up to 1.55‰ in dry soils and 0.49‰ for $\delta^{18}O$ for wet soils. In our study, chemical and salinity effects –which occur due to the fractionation of water molecules into hydration spheres around fully solvated cations compared to the pure water used to make the solutions –can be ignored for the silty sand due to a low CEC of 4.1 cmol(+) kg^{-1}. The high CEC (30.6 cmol(+) kg^{-1}) of the clayey loam soil may have caused some of the detrimental effects seen across laboratories. This is especially the case for low WCs due to ion hydration effects among the cations present (Table 2).

Gaj et al. (2017a) found out that the higher the abundance of Al_2O_3 or Fe_2O_3, commonly found in clay rich soils, the lower the ability to isotopically recover added water during spiking experiments. Our clayey loam contained 65% of SiO_2, but still 9% of Al_2O_3, which might have affected the obtained isotope ~~results~~ composition in general but cannot be an explanation for the high variability across labs.

~~Moreover, for environmental studies, the plant available water is of interest, which is not necessarily the same than the extracted water (Orlowski et al., 2018).~~

4.3 Differences between OA-ICOS- and IRMS-based measurements

Our OA-ICOS vs. IRMS comparison showed that isotope ~~results~~ data were significantly different between the two isotope measurement methods.

Others have found differences in isotope ~~results~~ data obtained from laser-based OA-ICOS and CRDS systems (cavity ring-down spectroscopy) in comparison to ~~and~~ IRMS isotope data (Martín-Gómez et al., 2015; Wassenaar et al., 2012). In a recently performed test, 235 international laboratories conducting water isotope analyses by OA-ICOS, CRDS and IRMS were evaluated. Wassenaar et al. (2018) could show that inaccuracy or imprecise performance stemmed mainly from skill- and knowledge-based errors including: calculation mistakes, inappropriate or compromised laboratory calibration standards, poorly performing instrumentation, lack of vigilance to contamination, or inattention to unreasonable isotopic outcomes. For the analysis of $\delta^{18}O$ and $\delta^{2}H$ via OA-ICOS, Penna et al. (2012) showed that between-sample memory effects can be an additional problem. Memory effect ranged from 14% and 9% for $\delta^{18}O$ and $\delta^{2}H$ measurements, respectively, but declined to 0.1% and 0.3% when the first ten injections of each sample were discarded.

An additional source of error in our study might be that sample preparation for water extraction was performed separately for OA-ICOS and IRMS analysis, but labs were instructed to follow the exact same procedure. Nevertheless, extractions were performed on independent samples, which might have led to differences in the extracts' isotope ~~results~~composition.

Leen et al. (2012) and West et al. (2010) have observed effects of co-extracted organic compounds leading to sample contamination. This can have a knock-on effect on isotope measurements via OA-ICOS. In our study, we found effects caused by organic contamination producing spectral interferences during OA-ICOS measurements (Fig. 7). This was mainly a problem for the clay soil water extracts, where we found narrow- and broad-band absorbers to be responsible for some of the outliers in the data sets. It did not seem to be a major issue for the silty sand soil water extracts. However, some labs applied longer extraction times to the clayey loam samples (see Fig. 2) which might have favored the co-extraction of organics. 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.

During an intercomparison water recovery experiment, Walker et al. (1994) faced difficulties to retrieve the added reference water from dry and wet clays, sand, and gypseous sand. They assumed that decomposition of organic matter or extraction of clay structural water could have caused isotope effects. Recently, Orlowski et al. (2016a) observed that δ^2H values correlated significantly, and became progressively lighter with increasing organic carbon content when using CWE. In environmental organic matter, the different existing exchangeable (i.e. labile) hydrogen fractions (O-, N-, and S-bonded or aromatic hydrogen) can easily interact with ambient water or water vapor (Ruppenthal et al., 2010) and thus are assumed to be the cause of the isotope effects.

Nevertheless, the less expensive, rapid option of the OA-ICOS is still a viable alternative for routine isotope analyses if no organic contamination issues are found and six or more injections are performed and the first two or more are discarded (Penna et al., 2012). If organics are present, proper correction schemes as per Martín-Gómez et al. (2015) need to be applied, especially when OA-ICOS data is used in ecohydrological studies. However, so far, correction procedures only account for contamination caused by methanol or ethanol but plant and soil water extracts can contain a variety of different contaminants. Our work showed that the silty sand soil water extracts were mainly free from organic contamination (Fig. 7). Still, data post-processing is highly recommended to detect issues occurring from co-extracted alcoholic compounds.

4.4 Take home messages about cryogenic water extraction

Our lab intercomparison did not find significant correlations between extraction condition parameters such as temperature, time, and applied vacuum, and the obtained isotope ~~results~~ data

(Fig. 2). Others have shown that extraction time and temperature have significant effects on the CWE isotope ~~results~~ data (Goebel and Lascano, 2012; Koeniger et al., 2011; Orlowski et al., 2013, 2016a; West et al., 2006). Gaj et al. (2017b) showed clear relationships between temperature and the release of water from interlayer cations and organics during CWE, which affected isotope ~~results~~values. They suggested using temperatures between 200°C and 300°C for clay water extractions. However, higher temperatures could cause a release of water by oxidation of organics and dihydroxylation of hydroxide-containing minerals, and the co-extraction of organics could become more important at harsher extraction conditions leading to spectral interferences when OA-ICOS is used. ~~this water might not be an ecohydrologically active part in the water cycle.~~ Orlowski et al. (2018) recently explored the effect of CWE for tracing plant source water. The authors tested the ability to match plant water to its putative soil water source(s) by using different CWE conditions (30–240 min, 80–200 °C, 0.1 Pa) for a clayey loam (same as in this study) and a sand. They showed that with higher extraction temperatures and longer extraction times, gradually more enriched soil water was extracted, which surprisingly reflected the plants' source water.

~~Moreover, the co-extraction of organics could become more important at harsher extraction conditions leading to spectral interferences when OA-ICOS is used.~~ Our interlab comparison was not able to provide any recommendations with regard to higher temperatures or longer extraction times leading to possibly better extraction results. Little is known about how the applied extraction pressure affects the CWE isotope ~~results~~data. But one thing is clear: that CWE is a 'brute force technique' (Orlowski et al., 2016a) in the sense that it is not able to distinguish between waters held at different soil tensions being of different importance for the ecohydrological water cycle. New instrumentation to sample discretely along the moisture release curve is desperately needed (McDonnell, 2014). For most past studies, possible fractionation effects associated with CWE remain unknown and the applied extraction parameters or cryogenic system specifications are often not indicated. Orlowski et al. (2018) recently stated that observed isotopic fractionation effects potentially lead to errors when CWE isotope data is used for plant water source calculation. This miscalculation in plant's water source could be quite large and could lead to misinterpretations of the role different plant species play in hydrologic processes at the ecosystem or larger scales. Millar et al. (2018) used the most common water extraction methods (centrifugation, microwave extraction, direct vapor equilibration, high-pressure mechanical squeezing, and two different CWE systems) for their intercomparison study on spring wheat (*Triticum aestivum L.*). The authors showed that all methods yielded markedly different isotopic signatures. The various methods also produced differing concentrations of co-extracted organic compounds. Again, CWE was outperformed by other extraction methods.

We found significant differences between extraction approach I (lab "in-house" procedure) and II (pre-defined extraction parameters). Both approaches showed significant differences to the added reference water for the OA-ICOS results, but in different ways. For example, for δ^2H signature recovery from silty sand, extraction approach II worked better. The same was true for δ^{18}O signature recovery for both WC treatments. However, for other settings, it was difficult to identify the ideal extraction approach that got closer to the reference DI water isotopic composition. We found no clear tendency for which approach should be applied, thus at present, and much to our dismay, we cannot define any standard protocol for CWE. 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.

We could show with our interlab comparison that a number of factors affect CWE results among which soil properties such as clay mineral composition and concomitant release of interlayer water seemed to be important. It is therefore essential to obtain detailed soil property information to be able to apply post-corrections as per Gaj et al. (2017a). Further research is urgently needed to analyze the full extent of soil organic matter effects (i.e. exchangeable bonded hydrogen (Meißner et al., 2014)) in organic-rich soils on the cryogenically extracted isotopic composition.

Future studies should test clay mineral fractionation effects on δ^{18}O and δ^2H during CWEs individually. We further recommend running individual CWE spiking tests on each natural soil material originating from field studies, also considering spatial variability of soil physicochemical properties over depth. Comparing the isotopic deviation of results from such spiking experiments with results from standardized soils will help to establish system-specific transfer functions. This will require considerable effort. However, it seems to be the only way to have some sort of calibration function for each extraction system and different soil types with their clay mineral composition.

5 Conclusions

This work presents results from a worldwide round robin laboratory intercomparison test of cryogenic extraction systems for soil water isotopic analysis. We tested the null hypothesis that with identical soils, standards, and isotope analyses, cryogenic extraction across laboratories should yield identical isotopic composition. The 16 participating laboratories used the same two standard soils along with reference water of known isotopic composition for CWEs. With our interlab comparison, we showed that multiple factors influence extracted isotopic signatures. Soil type, water content, as well as the applied type of isotope analysis (OA-ICOS vs. IRMS), showed major impacts, whereas, applied extraction parameters (time, temperature, and vacuum) interestingly did not affect CWE isotope ~~results~~ data across laboratories. Laboratory internal quality and water recovery rates showed additional effects.

Although the applied extraction system setups were different (e.g., size of extraction container, heating unit), we could not show a major impact of the system's design on the obtained isotope ~~results~~data, as laboratories were successful for the one soil type and water content but failed for the other. However, internal reproducibility ~~of isotope results~~ for the replicates of the same soil type at a given WC was given for most of the labs. Nevertheless, different results were obtained for $\delta^{18}O$ and $\delta^{2}H$.

Our intercomparison work showed that defining any sort of standard extraction procedure for CWEs across laboratories is challenging. Our results question the usefulness of this method as a standard for water extraction since results are not intercomparable across laboratories. A possible option might be that CWE labs establish system-specific calibration functions for each natural soil type, individually, to correct for the given offset to a set of reference soils.

New method intercomparison work on plant material showed that direct vapor equilibration is probably the most suitable extraction technique to be used when investigating plant water sourcing, at least for wheat. ~~Finally, we note that while CWEs for soils are problematic for reasons discussed in this paper, no work yet has seen any effects for plant water extractions apart from spectral interferences when using OA-ICOS. However~~However, ~~such~~ an inter-laboratory ~~and technique inter~~comparison is still lacking and should be addressed for plants in the future to account for possible effects. New continuous, in-situ measurements of soil and plant water isotopic composition might overcome isotope fractionation issues we observed with CWE.

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Tables

Table 1: Description of the respective extraction systems that participated in the cryogenic inter-laboratory comparison, ~~and~~ the applied extraction parameters for extraction approach I and the amount of sample material used for both extraction methods (lab-procedure: I and pre-defined: II)~~.~~ Note that not every lab provided the same detailed information.

Lab no.	Country	Description of CWE facility	Number of extraction slots	Extraction parameters for approach I	Amount of sample material used [g]
1	Germany	Similar to lab No. 8; pair of Valco Exetainer® vials connected with a 1.56 mm stainless steel capillary as extraction-collection unit; a hot plate, LN$_2$-cold trap	9	Temperature: 100°C, vacuum: 1-6 Pa, time: 60 min (silty sand) 120 min (clayey loam)	10-12
2	Canada	Mainly composed of different types of Swagelok® fittings (Swagelok Company, Solon, OH, US), flanges, and flexible hoses (Rettberg®, Rettberg Inc., Göttingen, DE), vacuum applied or shut off via diaphragm valves and monitored via DCP 3000 and VSK 3000 (Vacuubrand Inc., Wertheim, DE), glass tubes as extraction and collection units, LN$_2$-cold trap, water bath/sand bath	24	Temperature: on average 96°C, vacuum: 3.3-7.3 Pa, time: 90 min (silty sand) 240 min (clayey loam)	20
3	Germany	Heating lamps; LN$_2$-cold trap	5	Temperature: ~115°C, vacuum: 1 Pa, time: 90 min	20
4	Germany	A septum-sealed 70 mL vial (extraction) and a Valco Exetainer® vial (collection) connected with a stainless steel capillary as extraction-collection unit; heating block (aluminum), LN$_2$-cold trap	6	Temperature: 125°C, vacuum: 50 Pa, time: 33 min (silty sand at 8% WC) and 56 min (silty sand at 20% WC), 67 min (clayey loam at 8% WC), 83 min (clayey loam at 20% WC)	20
5	France	Cold trap: mixture of LN$_2$ and EtOH	4	Temperature: 65°C, cold trap: -50–-70°C, vacuum:	10

				0.1-1 Pa (static vacuum), time: 60-90 min	
6	Australia	Heating tape, glassware for extraction-collection unit; LN_2-cold trap	4	Temperature: 95-100ºC, starting with sealed vacuum of 0.3 Pa, time: 150-180 min	20
7	Chile	Heating element: reactor HI 839800 (Hanna instruments); size of extraction container: 22mL; precision measured with VD81 Thyracont model	9	Temperature: 105°C, vacuum: 12-23 Pa, time: 240 min	20
8	Germany	Pair of Valco Exetainer® vials connected with a 1.56 mm stainless steel capillary as extraction-collection unit; an aluminum block on a hot plate, LN_2-cold trap	12	Temperature: 200°C, vacuum: 50 Pa, time: 15 min	10
9	Germany	Stainless steel manifold (5 vials each), glass tubes as extraction-collection unit: 18 mm w, 150 mm l, LN_2-cold trap, water bath	20	Temperature: 95°C, vacuum: 0.8 Pa , time: 90 min	On average 43
10	Switzerland	Glass tubes (Vacutainer), LN_2-cold trap, water bath	20	Temperature: 80°C	Not specified
11	USA	Pyrex Culture Tubes (25mm x 150 mm), volume: 75 ml; heaters: electric coil (only allow to heat ⅔ of the tube)	10	Temperature: 102°C, vacuum: <0.1-2.7 Pa, time: on average 81 min (silty sand), 134 min (clayey loam)	10 for 20% WC, 20 for 8% WC
12	Germany	Glass tubes, LN_2-cold trap, water bath	8	Temperature: 80°C, vacuum: 600 Pa, time: 60 min	23
13	Germany	Glass tubes (Schott GL 18), LN_2-cold trap, sensor-regulated tube-shaped heating element	10	Temperature: 100°C, vacuum: 6.7-13.3 Pa, time: 15-266 min	10 for 20% WC, 20 for 8% WC
14	Germany	Glass tubes as extraction units, vacuum is generated by a Rotary vane pump (RZ 2.5, Vacuubrand, Wertheim,) and monitored via DCP 3000 with VSP 3000 (Vacuubrand), LN_2-cold trap, water bath	20	Temperature: 80°C, vacuum: 2-46 Pa, time: 30 min (silty sand), 40 min (clayey loam)	10
15	Germany	The septa of Labco exetainers® are pierced with a cannula (1.2 mm diameter) and connected to the vacuum system, vacuum is generated by	20	Temperature: 80°C, vacuum: 10-350 Pa, time: 30 min (silty sand), 40 min (clayey loam)	10

		a Rotary vane pump (RZ 2.5, Vacuubrand, Wertheim, Germany) and monitored via DCP 3000 with VSP 3000 (Vacuubrand), LN_2-cold trap, water bath			
16	Germany	Mainly composed of different types of Swagelok® fittings (Swagelok Company, Solon, OH, US), flanges, and flexible hoses (Rettberg®, Rettberg Inc., Göttingen, DE), vacuum applied or shut off via diaphragm valves and monitored via DCP 3000 and VSK 3000 (Vacuubrand Inc., Wertheim, DE), glass tubes as extraction and collection units, LN_2-cold trap, water bath/sand bath, high-purity nitrogen purging system	18	Temperature: 100°C, vacuum: 3.1-0.9 Pa, time: 45 min (silty sand), 240 min (clayey loam)	20

Table 2: Soil characteristics of clayey loam and silty sand (means ± SD). The clay mineral composition of soil samples was determined via X-ray powder diffraction (XRD, Philips X'Pert PW 1830 equipped with a PW2273/20 tube and a theta/theta-goniometer) following Poppe et al. (2016). Values were not corrected for reference intensity ratios (RIR). Alternating strata can occur for Illite/Smectite/Vermiculite. X-ray fluorescence (XRF) characterization of the chemical composition (in weight-%) was performed using an Axios spectrometer (PANalytical, EA Almelo, NL). Loss of ignition was 12.8 for the clayey loam and 1.3 for the silty sand.

Parameter	**Clayey loam**	**Silty sand**
pH-value	7.2 ± 0.2	5.0 ± 0.3
Water holding capacity [g 100g^{-1}]	43.4 ± 0.8	32.1 ± 1.4
Organic carbon [%]	2.0 ± 0.2	0.7 ± 0.1
Cation exchange capacity [cmol(+) kg^{-1}]	30.6 ± 5.1	4.1 ± 0.6
Particle size [mm] distribution according to German DIN [%]		
<0.002 (clay)	26	2.6
0.002–0.063 (silt)	46.4	12.7
0.063–2 (sand)	27.6	84.7
XRD analysis [relative %]		
Kaolinite	18.8	18.8
Illite	18	27.7
Chlorit	1.2	19.8
Vermiculite	43.4	2.9
Smectite	0.5	19.8
Mixed layered clays/alternating strata (Illite/Smectite/Vermiculite)	18.1	11.1
XRF analysis [%]		
SiO_2	65.1	92.3
TiO_2	0.4	0.1
Al_2O_3	8.8	3.3
Fe_2O_3	3.1	0.5
MnO	0.1	0.0
MgO	1.5	0.1
CaO	5.3	0.2
Na_2O	0.9	0.3
K_2O	1.7	1.7
P_2O_5	0.2	0.1
SO_3	0.1	<0.01
Cl	<0.002	<0.002
F	<0.05	<0.05

Figures

Figure 1. Water recovery rates (grouped from <80 to >98%) for both soil types (clayey loam and silty sand), WCs (8% and 20%) and OA-ICOS and IRMS (upper and lower panels, respectively) isotope ~~results~~ data in comparison to the spiked reference DI water (red asterisks) shown in dual isotope space. For reference, plots include the Global Meteoric Water Line (GMWL, solid red line). Water recovery rates are shown for those labs that provided the complete set of soil weight data (in % of previously added water).

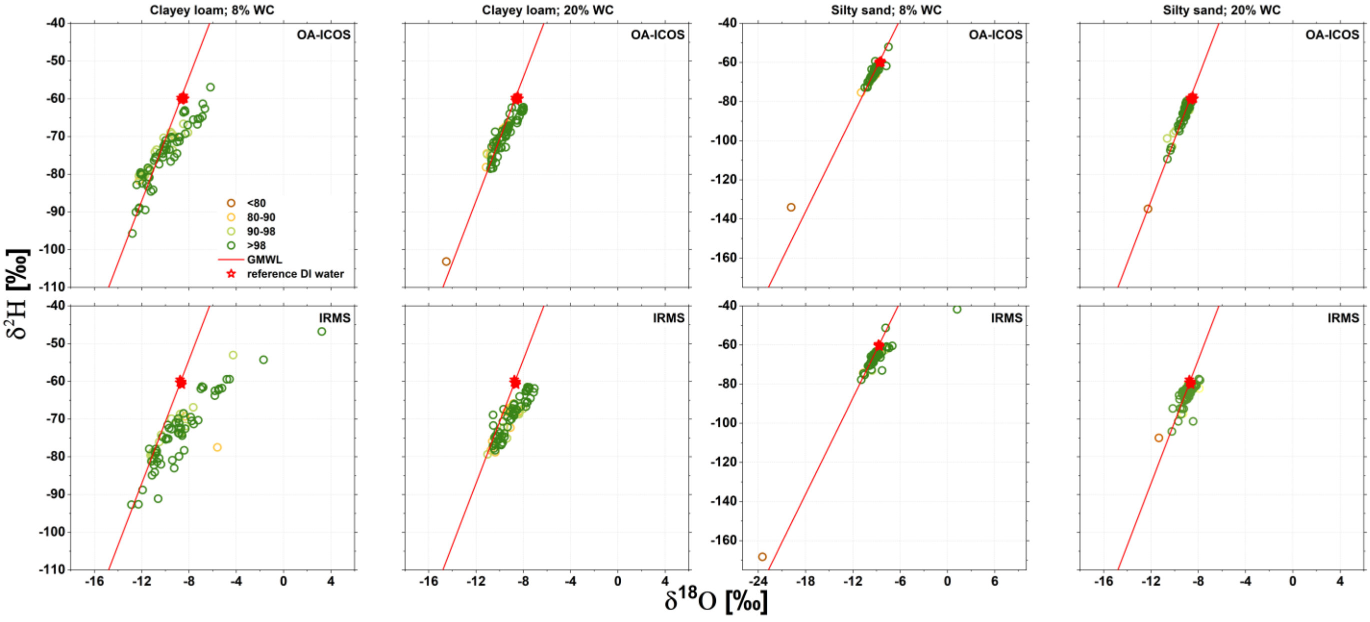


Figure 2. Effect of cryogenic extraction parameters (duration, temperature, and pressure) on δ^2H results of both soil types (clayey loam and silty sand) and WCs (8 and 20%) shown for all labs. The mean reference DI water δ^2H value is included as red dotted line.

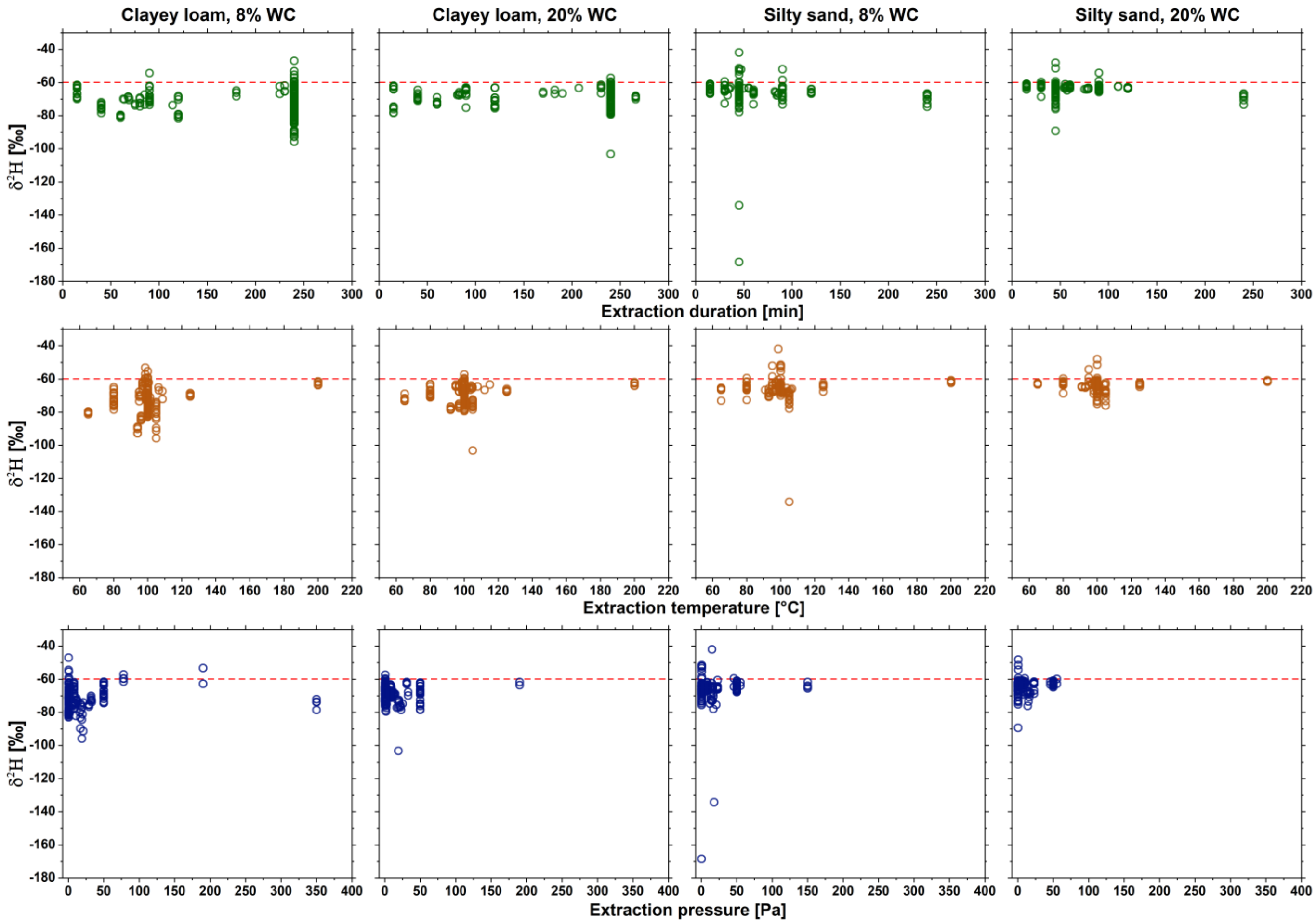


Figure 3. Mean differences from reference DI water for δ^2H OA-ICOS results of water extracts from both extraction methods (lab-procedure: I and pre-defined: II), soil types, and water contents (8 and 20% WC) including TSD of ±2 for δ^2H (Asterisk: -108.4 for δ^2H). Symbols represent the mean of the three replicates and y~~Y~~-error bars ~~represent~~ stand for the isotopic variation of the replicates. There were no significant differences between the two extraction approaches overall labs.

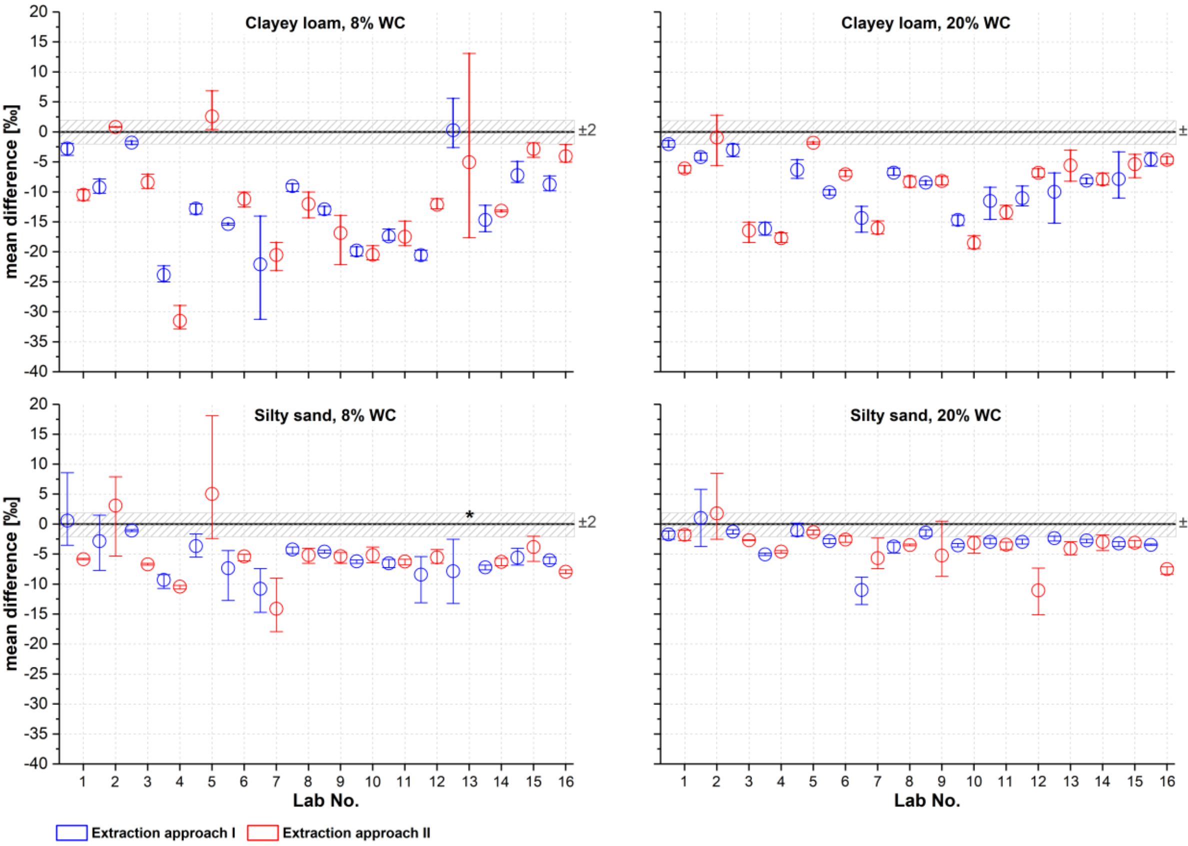


Figure 4. Mean differences from reference DI water for δ^{18}O OA-ICOS results of water extracts from both extraction methods (lab-procedure: I and pre-defined: II), soil types, and water contents (8 and 20% WC) including TSD of ±0.2 for δ^{18}O. Asterisks represent outliers. Symbols represent the mean of the three replicates and ~~Y~~y-error bars ~~represent~~ stand for the isotopic variation of the replicates. There were no significant differences between the two extraction approaches overall labs.

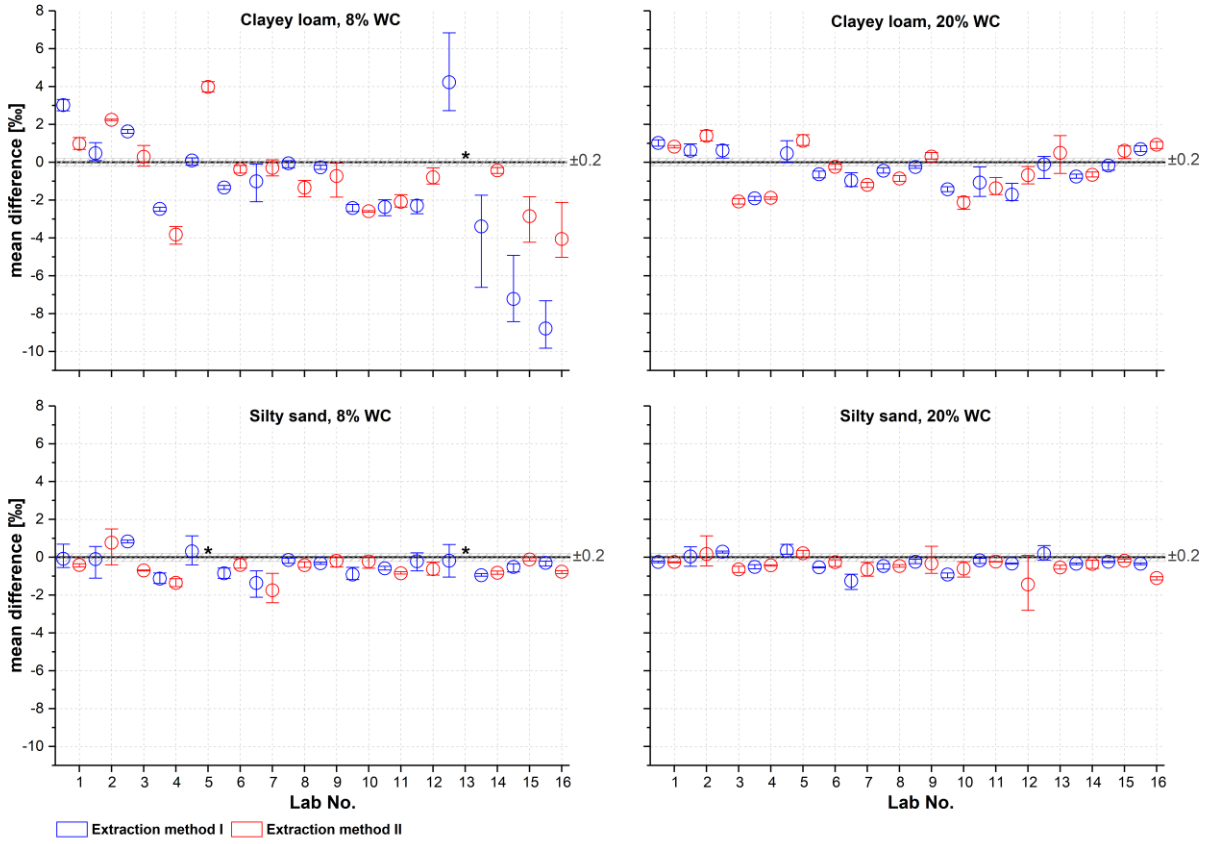


Figure 5. Dual isotope plots of clayey loam extracts for 8% and 20% WC in comparison to reference DI water (red asterisks) for OA-ICOS and IRMS data (upper and lower panels, respectively) from the 16 participating labs (different colors represent different labs) and both extraction methods (lab-procedure: I and pre-defined: II). For reference, plots include the Global Meteoric Water Line (GMWL, solid red line) and ~~evaporation~~ soil water regression lines for 8% and 20% WC (solid green and orange lines, respectively).

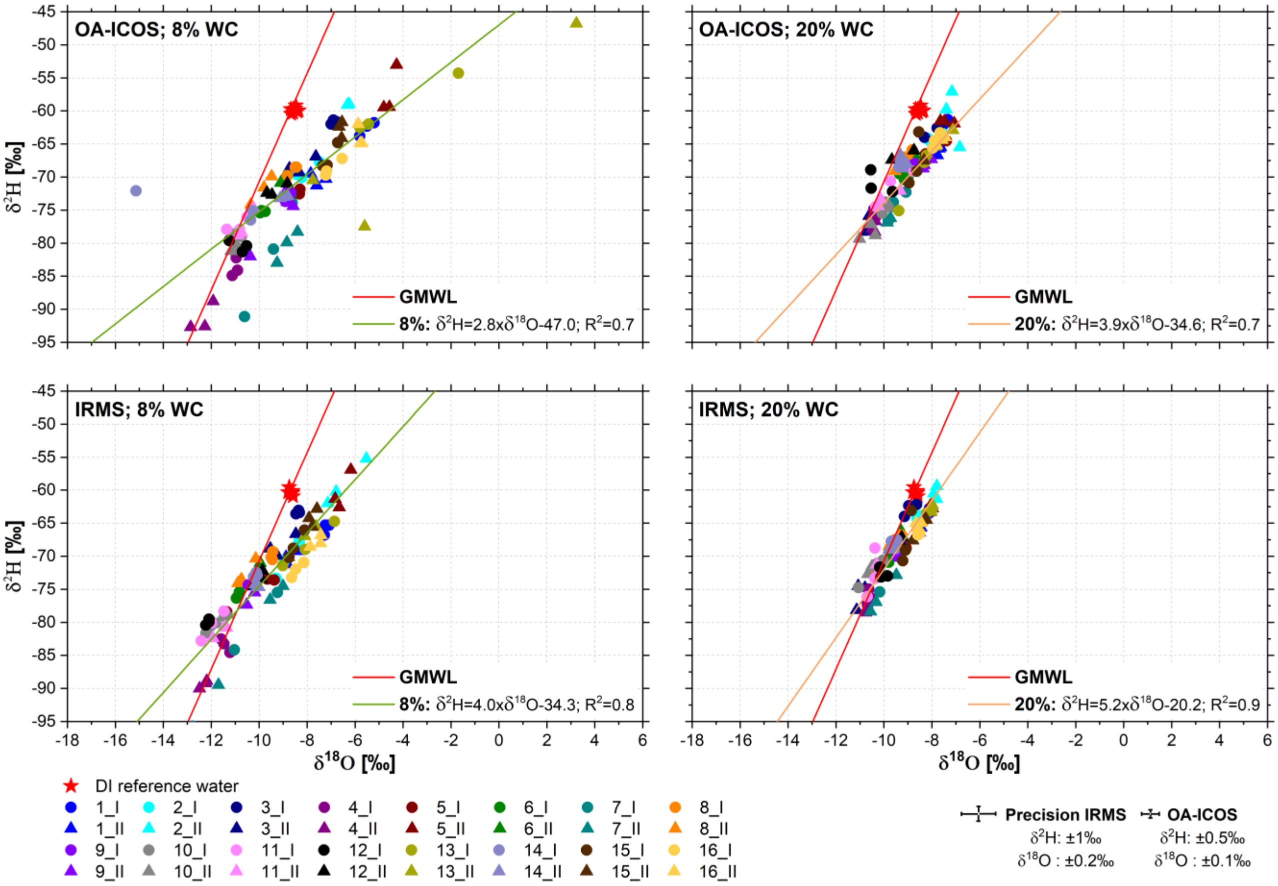


Figure 6. Dual isotope plots of silty sand extracts for 8% and 20% WC in comparison to reference DI water (red asterisks) for OA-ICOS and IRMS data (upper and lower panels, respectively) from the 16 participating labs (different colors represent different labs) and both extraction methods (lab-procedure: I and pre-defined: II). For reference, plots include the Global Meteoric Water Line (GMWL, solid red line) and ~~evaporation~~ soil water regression lines for 8% and 20% WC (solid green and orange lines, respectively).

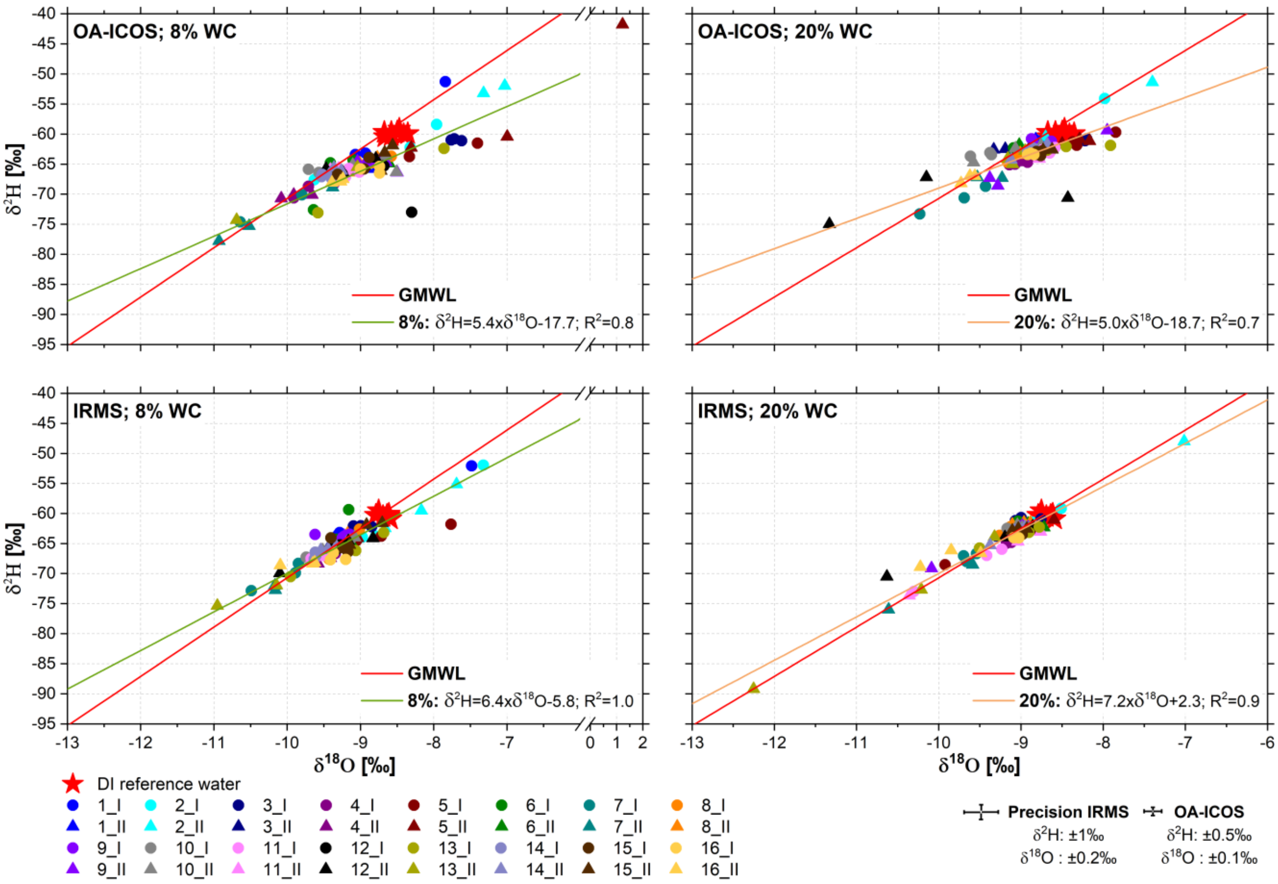
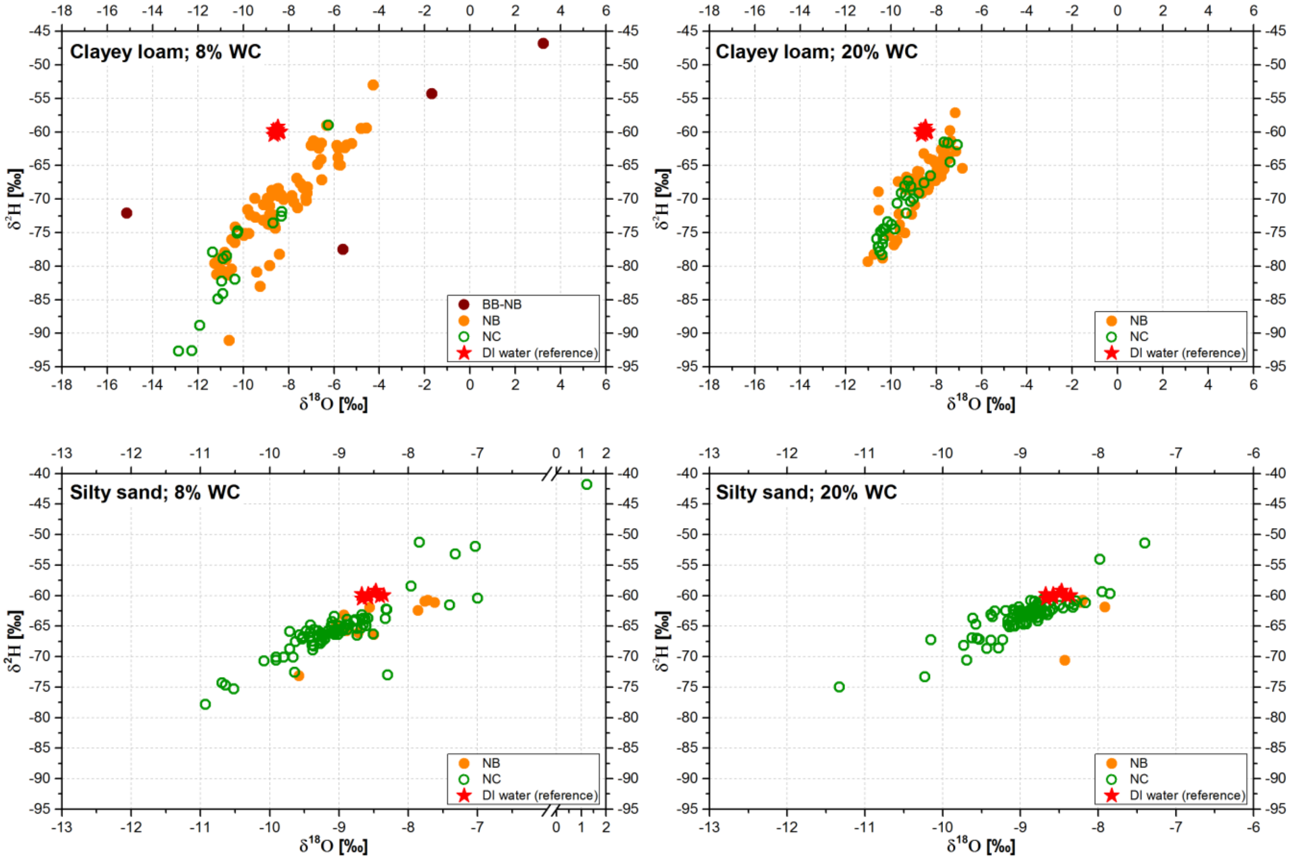


Figure 7. Dual isotope plots of clayey loam and silty sand extracts for 8% and 20% WC in comparison to reference DI water (red asterisks) for OA-ICOS analyses flagged by spectral contamination using the Spectral Contamination Identifier (LWIA-SCI) post-processing software (Los Gatos Research Inc.). BB-NB: Broad-and narrow-band absorbers (ethanol and methanol); NB: narrow-band absorber (methanol); NC: no contamination detected.



Appendix

Appendix 1. Cryogenic system – Questionnaire

Inter-laboratory comparison of CWE systems

Contact person

Last Name | First Name

Address

Street | Street No.

City | Postal Code | Country

Phone Number | Email

Cryogenic system – Questionnaire

How many numbers of extraction slots/units does your cryogenic extraction system have?

How much sample material (in gramm) is required for the cryogenic extraction at your system?

Does your laboratory have an operating procedure in terms of temperature, vacuum settings, and extraction times for soil and plant samples?

Do you have the possibility to adjust the extraction conditions (temperature, vacuum)?

To which type of sample material do you apply the cryogenic extraction method?

Type of plant material (e.g., twig, root crown) | Soil type

Please provide us a photo of your cryo-line.