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

Natalie Orlowski[a,b,l*], Lutz Breuer[b,c,], Nicolas Angeli[d], Pascal Boeckx[e], Christophe Brumbt[f], Craig S. Cook[g], Maren Dubbert[h], Jens Dyckmans[i], Barbora Gallagher[j], Benjamin Gralher[k], Barbara Herbstritt[l], Pedro Hervé-Fernández[e,f,m], Christophe Hissler[n], Paul Koeniger[o], Arnaud Legout[p], Chandelle Joan Macdonald[g], Carlos Oyarzún[f], Regine Redelstein[q], Christof Seidler[r], Rolf Siegwolf[s], Christine Stumpp[k,t], Simon Thomsen[u], Markus Weiler[l], Christiane Werner[h], and Jeffrey J. McDonnell[a]

[a] Global Institute for Water Security, School of Environment and Sustainability, University of Saskatchewan, Saskatoon, Canada

[b] Institute for Landscape Ecology and Resources Management (ILR), Research Centre for BioSystems, Land Use and Nutrition (IFZ), Justus Liebig University Giessen, Giessen, Germany

[c] Centre for International Development and Environmental Research, Justus Liebig University Giessen, Giessen, Germany

[d] INRA-UHP Ecologie et Ecophysiologie Forestières, INRA Centre de Nancy, Champenoux, France

[e] Isotope Bioscience Laboratory (ISOFYS), Faculty of Bioscience Engineering, University of Ghent, Ghent, Belgium

[f] Instituto de Ciencias de la Tierra, Universidad Austral de Chile, Valdivia, Chile

[g] Department of Ecosystem Science and Management, Stable Isotope Facility, University of Wyoming, Laramie, Wyoming, USA

[h] Chair of Ecosystem Physiology, University of Freiburg, Freiburg, Germany, BAYCEER; Chair of Ecosystem Physiology, University of Bayreuth, Bayreuth, Germany

[i] Institute of Soil Science and Forest Nutrition, Centre for Stable Isotope Research and Analysis (KOSI), University of Goettingen, Goettingen, Germany

[j] Institute for Environmental Research, Australia Nuclear Science and Technology Organization, Sydney, Australia

[k] Institute of Groundwater Ecology, German Research Center for Environmental Health, Helmholtz Zentrum München, Neuherberg, Germany

[l] Faculty of Environment and Natural Resources, Chair of Hydrology, Albert-Ludwigs University Freiburg, Freiburg, Germany

[m] Laboratory of Hydrology and Water management, Faculty of Bioscience Engineering, University of Ghent, Ghent, Belgium

[n] Luxembourg Institute of Science and Technology (LIST), Department of Environmental Research and Innovation (ERIN), Esch-sur-Alzette, Luxembourg, Luxembourg

[o] German Federal Institute for Geosciences and Natural Resources (BGR), Hannover, Germany

1 [p] INRA UR1138 Biogéochimie des Ecosystèmes Forestiers, INRA Centre de Nancy, Champenoux,
2 France

3 [q] Plant Ecology and Ecosystems Research, University of Goettingen, Goettingen, Germany

4 [r] Ecophysiology of Plants, Technical Universtiy Munich, Munich, Germany

5 [s] Stable Isotope Research Facility, Paul Scherrer Institute (PSI), Villigen, Switzerland

6 [t] Institute of Hydraulics and Rural Water Management (IHLW), University of Natural and Life
7 Sciences (BOKU) Vienna, Austria

8 [u] Institute of Soil Science, University of Hamburg, Hamburg, Germany

9 *Correspondence to: N. Orlowski, Natalie.Orlowski@hydrology.uni-freiburg.de

11 **Abstract**

12 For more than two decades, research groups in hydrology, ecology, soil science and
13 biogeochemistry have performed cryogenic water extractions for the analysis of δ^2H and $\delta^{18}O$
14 of soil water. Recent studies have shown that extraction conditions (time, temperature, and
15 vacuum) along with physicochemical soil properties may affect extracted soil water isotope
16 composition. Here we present results from the first worldwide round robin laboratory
17 intercomparison. We test the null hypothesis that with identical soils, standards, extraction
18 protocols and isotope analyses, cryogenic extractions across all laboratories are identical. Two
19 'standard soils' with different physicochemical characteristics along with deionized reference
20 water of known isotopic composition, were shipped to 16 participating laboratories. Participants
21 oven-dried and rewetted the soils to 8% and 20% gravimetric water content, using the deionized
22 reference water. One batch of soil samples was extracted via pre-defined extraction conditions
23 (time, temperature, and vacuum) identical to all laboratories; the second batch was extracted
24 via conditions considered routine in the respective laboratory. All extracted water samples were
25 analyzed for $\delta^{18}O$ and δ^2H by the lead laboratory (Global Institute for Water Security, GIWS,
26 Saskatoon, CA) using both a laser and an isotope ratio mass spectrometer (OA-ICOS and IRMS,
27 respectively). We rejected the null hypothesis. Our results showed large differences in retrieved
28 isotopic signatures among participating laboratories linked to soil type and soil water content
29 with mean differences to the reference water ranging from +18.1‰ to –108.4‰ for δ^2H and
30 +11.8‰ to –14.9‰ for $\delta^{18}O$ across all laboratories. In addition, differences were observed
31 between OA-ICOS and IRMS isotope data. These were related to spectral interferences during
32 OA-ICOS analysis that are especially problematic for the clayey loam soils used. While the
33 types of cryogenic extraction lab construction varied from manifold systems to single chambers,

1 no clear trends between system construction, applied extraction conditions, and extraction

2 results were found. Rather, observed differences in the isotope data were influenced by

3 interactions between multiple factors (soil type and properties, soil water content, system setup,

4 extraction efficiency, extraction system leaks, and each lab's internal accuracy). Our results

5 question the usefulness of cryogenic extraction as a standard for water extraction since results

6 are not comparable across laboratories. This suggests that defining any sort of standard

7 extraction procedure applicable across laboratories is challenging. Laboratories might have to

8 establish calibration functions for their specific extraction system for each natural soil type,

9 individually.

11   1 Introduction

12 The interpretation of the stable isotope signatures of water (δ^2H and δ^{18}O) from soils in many

13 research disciplines relies on accurate, high-precision measurements (Wassenaar et al., 2012).

14 To extract water from soils for isotopic analysis, cryogenic water extraction (CWE) is the most

15 widely used laboratory-based removal technique (Araguás-Araguás et al., 1995; Orlowski et

16 al., 2016a). The ability to obtain measurable amounts of water from small sample sizes

17 (i.e. < 10 g) makes this method attractive. However, CWE is also accompanied by high capital

18 and operating costs. Despite its widespread use, recent work has identified several extraction

19 artifacts leading to uncertain isotopic signature identification (Gaj et al., 2017a; Orlowski et al.,

20 2016b). Studies have shown that extraction conditions (i.e., extraction time, temperature, and

21 vacuum) need to be adapted specifically to the soil used (Araguás-Araguás et al., 1995; Gaj et

22 al., 2017a; Meißner et al., 2014; Orlowski et al., 2016a). Notwithstanding, isotope effects

23 triggered by physicochemical soil properties (e.g., clay minerals, soil organic carbon content,

24 and water content) can occur (Araguás-Araguás et al., 1995; Gaj et al., 2017a; Meißner et al.,

25 2014; Oerter et al., 2014; Orlowski et al., 2013). However, the ecohydrology and soil science

26 communities currently lack clear recommendations for standardized water extraction conditions

27 from soils. Although there seems to be an agreement on the need to control the extraction yield

28 of cryogenic extraction facilities (recovery rate in percentage of previously added water), there

29 exists a large variability in the applied extraction conditions between laboratories. Moreover,

30 extraction systems vary in terms of heating elements, size of extraction containers, or

31 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 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

1 loam and silty sand) from the German State Research Institute for Agriculture (LUFA Speyer,

2 2015) (Table 2) were used for the interlaboratory comparison.

4 [Table 2 near here]

6 We chose a silty sand from which we expected water extractions to be relatively easy for each

7 laboratory without cation ion exchange problems, and a clayey loam soil,—which is known to

8 be challenging for CWE extraction systems. Clayey soils can be difficult due to interactions

9 with the clay fraction and different types of clay minerals—the so-called adsorbed cation effect

10 (Oerter et al., 2014). Clay soils also present challenges with regard to the tightness of water

11 bound to mineral surfaces which causes an additional isotope effect (Ingraham and Shadel,

12 1992; Oerter et al., 2014; Walker et al., 1994).

13 Soil samples were sieved to a grain size <2 mm. Soils were pre-dried at 105°C for 48 h,

14 homogenized, and shipped in tightly sealed glass bottles to the 16 independent laboratories

15 along with deionized (DI) reference water of known isotopic composition (measured on both

16 an IWA-45EP Analyzer (OA-ICOS, Los Gatos Research Inc., Mountain View, US): δ^2H:

17 -59.8 ± 0.2‰ and δ^{18}O: -8.5 ± 0.1‰, n=6; and via Delta V™ Advantage mass spectrometer

18 (Thermo Fisher Scientific, Waltham, MA, US): δ^2H: -60.5 ± 0.2‰ and δ^{18}O: -8.7 ± 0.1‰, n=6).

19 All bottles containing either soils or DI water were filled, capped tightly, and wrapped with

20 Parafilm® to prevent water loss. We decided not to ship ready-to-use rehydrated soils to avoid

21 evaporation fractionation effects and to give participants the opportunity to adjust e.g. samples

22 sizes to the specific requirements of their extraction system. Water loss and evaporative

23 enrichment from the shipped DI water was checked by isotopic comparison of shipped and non-

24 shipped DI water (1. shipment test: Giessen–Freiburg (Germany)–Saskatoon (Canada) and 2.

25 shipment test: Giessen (Germany)–Saskatoon (Canada) vs. non-shipped water samples). After

26 this simple experiment, isotope fractionation effects due to shipment were excluded.

27 As a reliability test, each participant in the intercomparison performed water cryogenic

28 extractions (defined here as simply extracting pure water, i.e. without any soil material present)

29 using their extraction facility. This was done in order to determine the capability of the

30 respective extraction apparatus to recapture water of known isotopic composition. After

31 showing the operational reliability, CWEs with the rehydrated soil samples were performed

32 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. 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 follow two different extraction approaches: (I) For the first subset of rehydrated soil samples, participants applied the CWE procedure considered routine

1 in their laboratory for the specific soil type and soil water content. (II) With the second subset,

2 CWE under pre-defined conditions for all labs was performed: For silty sand, a 45 min

3 extraction time was used while 240 min was applied to clayey loam samples, both at an

4 extraction temperature of 100°C and a vacuum of 0.3 Pa. These pre-defined extraction

5 parameters were identical for all participating laboratories. For comparison, in past studies

6 extraction times from 2.5 min (Koeniger et al., 2011) over 30 min (West et al., 2006) to 7 h

7 (Araguás-Araguás et al., 1995) for sandy soils and from 30 min (Goebel and Lascano, 2012)

8 over 40 min (West et al., 2006) to 8 h (Araguás-Araguás et al., 1995) for clayey soils were

9 reported.

10 Three replicates per soil type and soil water content resulting in 24 samples per extraction

11 procedure (pre-defined and laboratory specific) and isotope analysis method (OA-ICOS and

12 IRMS) were processed (n=48 in total). Pre- and post-oven-dried (105°C for 24 h) soil sample

13 weights were used to determine water recovery rates. All extracted water samples were

14 transferred to 2 mL amber glass vials capped with solid lids (Th. Geyer Inc., Renningen, DE),

15 tightly sealed with Parafilm®, labeled, and shipped to the GIWS for isotope analysis. If the

16 amount of extracted water was not sufficient to entirely fill the 2 mL vial, inserts (0.2 mL) were

17 used (Th. Geyer Inc., Renningen, DE) to minimize sample vial headspace, following standard

18 procedures as outlined by the IAEA (2014).

20 **2.4 Isotope analyses**

21 For cross-checking isotope data and ruling out potential lab analytical differences, the isotopic

22 composition of the extracted water samples was analyzed via both OA-ICOS and IRMS. OA-

23 ICOS samples were analyzed on an IWA-45EP Analyzer (Los Gatos Research Inc., Mountain

24 View, US). The accuracy of OA-ICOS analyses was ±0.5‰ for δ^2H and ±0.1‰ for $\delta^{18}O$. IRMS

25 samples were analyzed on a Delta V™ Advantage mass spectrometer (Thermo Fisher

26 Scientific, Waltham, MA, US) and an H/Device peripheral using a Cr-reduction method for

27 δ^2H analysis (Morrison et al., 2001). For $\delta^{18}O$ analysis, a GasBench II peripheral was utilized.

28 Using mass spectrometry, a conversion from the water into a light gas suitable for mass

29 spectrometry (H_2, CO_2, CO, O_2) is necessary. This conversion step often turns out to limit the

30 achievable precision of IRMS (Brand et al., 2009). In our case, IRMS results are accurate to

31 ±1‰ for δ^2H and to ±0.2‰ for $\delta^{18}O$, respectively. All isotope ratios are reported in per mil (‰)

32 relative to Vienna Standard Mean Ocean Water (VSMOW) (δ^2H or

1 $\delta^{18}O=(R_{sample}/R_{standard}-1)\times1000‰)$, where R is the isotope ratio of the sample and the known

2 reference (i.e. VSMOW)) (Craig, 1961). In-house standards, calibrated against VSMOW2 and

3 SLAP2, were run as samples to allow the results to be reported against VSMOW (Nelson,

4 2000).

5 OA-ICOS isotope data of soil water extracts were checked but not corrected for spectral

6 interferences (caused by potentially co-extracted organics such as methanol or ethanol) using

7 the Spectral Contamination Identifier post-processing software (LWIA-SCI, Los Gatos

8 Research Inc.) when measured via OA-ICOS. This software compares recorded spectra from

9 unknown samples with those from known non-contaminated samples (standards) to produce a

10 metric of contamination from either narrow-band (e.g., methanol (MeOH)) or broad-band (e.g.,

11 ethanol (EtOH)) absorbers which indicates the likelihood or degree of spectral interference

12 (Schultz et al., 2011). IRMS results are generally not affected by organic contaminants.

14 **2.5 Statistical evaluation**

15 We used R for statistical analyses (R version 3.3.2; R Core Team, 2014). For quantifying

16 laboratory variances, differences between pre-defined and laboratory specific extraction

17 procedures, effects of soil type and WC, differences between OA-ICOS and IRMS, all data

18 were tested for normality using the Shapiro-Wilk test. Homoscedasticity was tested using either

19 the Levene's test for normally distributed data or the Fligner-Killeen test for non-normally

20 distributed data. Cook's distance was determined in order to identify outliers (D>1). Depending

21 on the type of data (normally distributed and homoscedastic), either Kruskal-Wallis rank sum

22 tests or Analyses of Variances (ANOVAs) were applied and posthoc tests (e.g., Nemenyi-tests)

23 were run to determine which groups were significantly different ($p\leq0.05$). P-value adjustments

24 via the FDR-method (false discovery rate) were applied to reduce the family-wise-error rate

25 (Zieffler et al., 2012).

26 For graphical comparisons, a target standard deviation (TSD) for acceptable performance was

27 set to ±2‰ for δ^2H and ±0.2‰ for $\delta^{18}O$ similar to Orlowski et al. (2016b), which is considered

28 reasonable for hydrologic studies (Wassenaar et al., 2012). The TSD does not account for errors

29 associated with the extraction method itself, weighing errors, and volumetric water additions to

30 the sample, or any standard deviations (1SDs) related to the isotope analysis. Statistically

31 significant ($p\leq0.05$) linear regressions were added to dual isotope plots as references as well as

1   the Global Meteoric Water Line (GMWL: $\delta^2H=8.2\times \delta^{18}O + 11.3‰$, as defined by Rozanski et

2   al. (1993)).

4   **3 Results**

5   **3.1 Cryogenic extraction systems and water extraction efficiencies**

6   Cryogenic extraction systems varied greatly from lab to lab: from manifold, high-throughput

7   devices (as described by Orlowski et al. (2013)) to small, single chamber systems (as in

8   Koeniger et al. (2011) and West et al. (2006)) (for details see Table 1). The systems showed

9   differences in terms of the extraction containers (form, size, volume, and material), the heating

10   module and its application temperature (heating tapes or lamps, water baths or hot plates), the

11   type of fittings and connections (glass, stainless steel), as well as in the vacuum producing units

12   (Table 1). In relation to the amount of used sample material, most labs either introduced 10 or

13   20 g to their system no matter the extraction approach (I or II), soil type or WC. Only labs 11

14   and 13 chose different weights with respect to the WC, e.g., 10 g for the higher WC (20%) and

15   20 g for 8% WC for extraction approach I.

16   To determine the degree of extraction efficiency for each lab's samples, water recovery rates

17   were calculated for those labs that provided the complete set of soil weight data (in % of

18   previously added water). When comparing water recovery rates against δ^2H and $\delta^{18}O$ values,

19   the clayey soil showed no clear trend (Fig. 1). Even if water recovery rates were higher than

20   98% (following the definition of (Araguás-Araguás et al., 1995)), extracted isotope values

21   differed from the reference DI water (Fig. 1). For example, at 8% soil water content (WC),

22   recovery rates of above 98% were achieved, but isotope values were depleted in comparison to

23   the reference DI water (Fig. 1, left panels).

25   [Figure 1 near here]

27   For the silty sand, recovery rates were generally higher in comparison to the clayey soil. Only

28   a few samples showed extraction efficiencies lower than 98% (Fig. 1, right panels).

29   Surprisingly, we observed some recovery rates higher than 100%. This was especially an issue

30   for soils at 8% WC (Fig. 1).

31   Correlation analysis was performed in order to relate extraction parameters (i.e., time,

32   temperature or vacuum) to OA-ICOS and IRMS isotope data.

2   [Figure 2 near here]

4   We found no significant correlations between the extraction parameters and the respective

5   isotope data, exemplarily shown for δ^2H results (Fig. 2) (e.g., R^2=0.0 for δ^2H vs. duration or

6   temperature).

8   **3.2 Laboratory performance with respect to water content and soil type**

9   Figures 3 and 4 show the mean differences between the extracted samples via the lab

10 procedure's extraction approach (I) and the pre-defined extraction approach (II) and the

11 reference DI water δ^2H and δ^{18}O values, respectively.

12 For the 8% WC tests, mean differences for the clayey loam ranged from +13.1 to −32.8‰ for

13 δ^2H. For the individual lab procedure's extraction approach (I) at 8% WC for the clayey loam,

14 two laboratories (lab 3 and 8) were able to get back to the reference δ^2H value based on no

15 statistically significant differences (p>0.05) (Fig. 3, upper left plot). For the pre-defined

16 extraction approach (II) at 8% WC, two other labs recovered the δ^2H value from the clayey

17 loam (lab 9 and 15).

18 For soil samples with 20% WC, variation among laboratories was smaller but only one

19 laboratory (lab 9) recovered the reference DI water δ^2H value applying the pre-defined

20 extraction approach for the clayey loam. Mean differences between the clayey loam extracts

21 and the reference DI water ranged from +2.8 to −19.5‰ (Fig. 3, upper right plot).

23 [Figure 3 near here]

25 Mean differences between the silty sand water extraction and the reference δ^2H signature were

26 in a smaller range of ±18‰ than clayey loam extracts from the same treatment (8% WC).

27 For the individual lab procedure's extraction approach (I) at 8% WC, five laboratories recovered

28 the added label from the silty sand (Fig. 3, lower left plot) with no statistical differences between

29 the reference DI water (p>0.05) (labs 6, 8, 9, 13, and 15), whereas for the pre-defined extraction

30 approach (II) at 8% WC, three labs got back to the added δ^2H value (labs 9, 12, and 15).

31 For silty sand at 20% WC, most laboratories' results even fell close to the range of the TSD of

32 ±2‰. Mean differences to the reference DI water δ^2H signature ranged from +8.5 to −15.1‰

1 (Fig. 3, lower right plot). However, extraction approach I was statistically not successful in

2 recovering the added label ($p<0.05$), but five laboratories (6, 9, 10, 14, and 15) showed no

3 significant differences to the reference DI water when applying extraction approach II to the

4 silty sand at 20% WC.

5 Laboratories performed better for $\delta^{18}O$ signature recovery, especially with extraction approach

6 I. For both clayey loam WC treatments, labs 13 and 15 were the most successful. Again, mean

7 differences to the reference DI water were larger for the 8% WC than for the 20% WC (Fig. 4,

8 upper plots). However, for the clayey loam at 20% WC with the pre-defined approach (II) only

9 lab 13 and 14 did not show statistically significant differences to the added $\delta^{18}O$ signature

10 (Figure 4, upper right plot) ($p>0.05$).

12   [Figure 4 near here]

14 For the silty sand, most laboratories were able to get back the known value with no statistically

15 significant differences to the reference $\delta^{18}O$ value (Fig. 4, lower plots). For both WC treatments

16 of the silty sand, extraction approach II seemed to work better in recovering the added label.

18 Across both soil types, WC treatments, and extraction approaches, lab 13 was the most

19 successful in recovering the reference $\delta^{18}O$ value, whereas for δ^2H recovery lab 9 gained back

20 the added label in most of the cases.

21 In general, δ^2H and $\delta^{18}O$ values were neither comparable between laboratories nor between one

22 laboratory at different soil types or WCs, meaning that a specific laboratory, for example,

23 successfully recovered the added DI water value for silty sand but was not able to gain back the

24 known label for clayey loam. Moreover, recovery results differed between both isotopes. For

25 example, lab 13 was the most successful for $\delta^{18}O$ but not for δ^2H signature recovery. In terms

26 of lab internal reproducibility, some labs showed small standard deviations for the replicates of

27 the same soil type at a given WC (Fig. 3 and 4); even so, resulting isotope values differed

28 statistically significant from the introduced reference DI water.

30 **3.3 Differences between OA-ICOS- and IRMS-based measurements**

31 Figures 5 (clayey loam) and 6 (silty sand) illustrate data variability for each laboratory and WC

32 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≤0.05). 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^2H 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^2H measured via OA-ICOS and ±4.2 for IRMS, respectively). However, those data sets still did not reach the TSD of ±2‰ for δ^2H and ±0.2‰ for δ^{18}O.

[Figure 5 near here]

For comparison, apart from 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, 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 water regression lines showed greater slopes (5.4–7.2 across both WCs and isotope analysis) than clayey loam's soil water regression lines (2.8–5.2 across both WCs and isotope analysis) (Figures 5 and 6). The clayey loam 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 with a lower slope relative to the original water (Gonfiantini, 1986). 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 $\delta^{18}O$) of the added reference DI water. Some laboratories were able to get back to the reference δ^2H value but were not successful for $\delta^{18}O$.

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

19 It is also possible that participating labs did not follow the pre-defined extraction procedure (II)
20 in the exactly same ways. Even extraction results from some individual labs for the same soil
21 type and WC showed high SDs (Fig. 3 and 4) which questions the overall repeatability of
22 individual water extraction results. For the first, "in-house" extraction approach (I), not all
23 laboratories indicated the precise extraction conditions (extraction temperature, time, and
24 vacuum) that they used for the specific soil types and WCs.
25 As an additional performance test, laboratories were asked to perform simple water extractions
26 to show their ability to recover water of known isotopic composition prior to soil-based tests.
27 For example, some laboratories, like lab 2, showed a high accuracy for these water extractions
28 of $\pm 0.4‰$ for δ^2H and $\pm 0.1‰$ for $\delta^{18}O$ (n=119) as well as lab 16. They performed extraction
29 tests with tap water, which resulted in no significant differences between the initial, untreated
30 ($-56.7‰ \pm 0.4$ for δ^2H and $-9.3‰ \pm 0.1$ for $\delta^{18}O$) and extracted tap water ($-57.5‰ \pm 0.6$ for
31 δ^2H and $-9.4‰ \pm 0.1$ for $\delta^{18}O$). These examples show that these labs among others were able

to reach the TSD with simple 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 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) 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 $\delta^{18}O$) 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 isotope 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 composition in general but cannot be an explanation for the high variability across labs.

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

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

Others have found differences in isotope data obtained from laser-based OA-ICOS and CRDS systems (cavity ring-down spectroscopy) in comparison to 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 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 data (Fig. 2). Others have shown that extraction time and temperature have significant effects on the CWE isotope 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 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. 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.

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 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 $\delta^{18}O$ 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 $\delta^{18}O$ 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 data across laboratories. Laboratory internal quality and water recovery rates showed additional effects.

1   Although the applied extraction system setups were different (e.g., size of extraction container,

2   heating unit), we could not show a major impact of the system's design on the obtained isotope

3   data, as laboratories were successful for the one soil type and water content but failed for the

4   other. However, internal reproducibility for the replicates of the same soil type at a given WC

5   was given for most of the labs. Nevertheless, different results were obtained for $\delta^{18}O$ and δ^2H.

6   Our intercomparison work showed that defining any sort of standard extraction procedure for

7   CWEs across laboratories is challenging. Our results question the usefulness of this method as

8   a standard for water extraction since results are not intercomparable across laboratories. A

9   possible option might be that CWE labs establish system-specific calibration functions for each

10   natural soil type, individually, to correct for the given offset to a set of reference soils.

11   New method intercomparison work on plant material showed that direct vapor equilibration is

12   probably the most suitable extraction technique to be used when investigating plant water

13   sourcing, at least for wheat. However, an inter-laboratory comparison is still lacking and should

14   be addressed for plants in the future to account for possible effects. New continuous, in-situ

15   measurements of soil and plant water isotopic composition might overcome isotope

16   fractionation issues we observed with CWE.

1 **Acknowledgements**

2 This interlaboratory test would not have been possible without the generous cooperation of the

3 researchers and technical staff in our 16 stable water isotope laboratories. We especially thank

4 Kim Janzen, Cody Millar, and Anna Winkler for their laboratory-support and Nathalie Steiner

5 for statistical support. The Gibson laboratory from Alberta Innovates Technology Futures is

6 thanked for IRMS analyses. This research was supported by an NSERC Discovery Grant and

7 Accelerator Award to J. J. McDonnell.

References

Aggarwal, P. K., Dillon, M., A. and Tanweer, A.: Isotope fractionation at the soil-atmosphere interface and the [18]O budget of atmospheric oxygen, Geophys. Res. Lett., 31(14), 1–4, doi:10.1029/2004GL019945, 2004.

Araguás-Araguás, L., Rozanski, K., Gonfiantini, R. and Louvat, D.: Isotope effects accompanying vacuum extraction of soil water for stable isotope analyses, J. Hydrol., 168(1–4), 159–171, doi:10.1016/0022-1694(94)02636-P, 1995.

Benettin, P., Volkmann, T. H. M., von Freyberg, J., Frentress, J., Penna, D., Dawson, T. E. and Kirchner, J. W.: Effects of climatic seasonality on the isotopic composition of evaporating soil waters, Hydrol Earth Syst Sci, 22(5), 2881–2890, doi:10.5194/hess-22-2881-2018, 2018.

Brand, W. A., Geilmann, H., Crosson, E. R. and Rella, C. W.: Cavity ring-down spectroscopy versus high-temperature conversion isotope ratio mass spectrometry; a case study on δ^2H and δ^{18}O of pure water samples and alcohol/water mixtures, Rapid Commun. Mass Spectrom., 23(12), 1879–1884, doi:10.1002/rcm.4083, 2009.

Craig, H.: Standard for Reporting Concentrations of Deuterium and Oxygen-18 in Natural Waters, Science, 133(3467), 1833–1834, doi:10.1126/science.133.3467.1833, 1961.

Gaj, M., Kaufhold, S., Koeniger, P., Beyer, M., Weiler, M. and Himmelsbach, T.: Mineral mediated isotope fractionation of soil water, Rapid Commun. Mass Spectrom., 31(3), 269–280, doi:10.1002/rcm.7787, 2017a.

Gaj, M., Kaufhold, S. and McDonnell, J. J.: Potential limitation of cryogenic vacuum extractions and spiked experiments, Rapid Commun. Mass Spectrom., doi:10.1002/rcm.7850, 2017b.

Goebel, T. S. and Lascano, R. J.: System for high throughput water extraction from soil material for stable isotope analysis of water, J. Anal. Sci. Methods Instrum., 02(04), 203–207, doi:10.4236/jasmi.2012.24031, 2012.

Gonfiantini, R.: Environmental isotopes in lake studies, in Handbook of environmental isotope geochemistry: The terrestrial environment, B, edited by P. Fritz and J. C. Fontes, pp. 113–168, Elsevier, New York, USA., 1986.

Grim, R. and Bradley, W.: Investigation of the Effect of Heat on the Clay Minerals Illite and Montmorillonite, J. Am. Ceram. Soc., 23(8), 242-248., doi:10.1111/j.1151-2916.1940.tb14263.x, 1940.

IAEA (International Atomic Energy Agency): IAEA/GNIP precipitation sampling guide, http://www-naweb.iaea.org/napc/ih/documents/other/gnip_manual_v2.02_en_hq.pdf (Accessed 15 June 2014), 2014.

Ingraham, N. L. and Shadel, C.: A comparison of the toluene distillation and vacuum/heat methods for extracting soil water for stable isotopic analysis, J. Hydrol., 140(1–4), 371–387, doi:10.1016/0022-1694(92)90249-U, 1992.

1 Kaufhold, S. and Dohrmann, R.: Comparison of the traditional Enslin-Neff method and the
2 modified dieng method for measuring water-uptake capacity, Clays Clay Miner., 56(6), 686-
3 692., doi:10.1346/CCMN.2008.0560609, 2008.

4 Koeniger, P., Marshall, J. D., Link, T. and Mulch, A.: An inexpensive, fast, and reliable method
5 for vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry,
6 Rapid Commun. Mass Spectrom., 25(20), 3041–3048, doi:10.1002/rcm.5198, 2011.

7 LUFA Speyer: German State Research Institute for Agriculture, Speyer, DE, http://www.lufa-
8 speyer.de/index.php/dienstleistungen/standardboeden/8-dienstleistungen/artikel/57-standard-
9 soils (Accessed 15 April 2015), 2015.

10 Martín-Gómez, P., Barbeta, A., Voltas, J., Peñuelas, J., Dennis, K., Palacio, S., Dawson, T. E.
11 and Ferrio, J. P.: Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of
12 plant-water sources?, New Phytol., 1–14, doi:10.1111/nph.13376, 2015.

13 McDonnell, J. J.: The two water worlds hypothesis: ecohydrological separation of water
14 between streams and trees?, Wiley Interdiscip. Rev. Water, 1(4), 323–329,
15 doi:10.1002/wat2.1027, 2014.

16 Meißner, M., Köhler, M., Schwendenmann, L., Hölscher, D. and Dyckmans, J.: Soil water
17 uptake by trees using water stable isotopes (δ^2H and δ^{18}O)−a method test regarding soil
18 moisture, texture and carbonate, Plant Soil, 376(1–2), 327–335, doi:10.1007/s11104-013-1970-
19 z, 2014.

20 Millar, C., Pratt, D., Schneider, D. J. and McDonnell, J. J.: A comparison of extraction systems
21 for plant water stable isotope analysis, Rapid Commun. Mass Spectrom., 32(13), 1031–1044,
22 doi:10.1002/rcm.8136, 2018.

23 Morrison, J., Brockwell, T., Merren, T., Fourel, F. and Phillips, A. M.: On-line high-precision
24 stable hydrogen isotopic analyses on nanoliter water samples, Anal. Chem., 73(15), 3570–3575,
25 2001.

26 Nelson, S. T.: A simple, practical methodology for routine VSMOW/SLAP normalization of
27 water samples analyzed by continuous flow methods, Rapid Commun. Mass Spectrom., 14(12),
28 1044–1046, doi:10.1002/1097-0231(20000630)14:12<1044::AID-RCM987>3.0.CO;2-3,
29 2000.

30 Oerter, E., Finstad, K., Schaefer, J., Goldsmith, G. R., Dawson, T. and Amundson, R.: Oxygen
31 isotope fractionation effects in soil water via interaction with cations (Mg, Ca, K, Na) adsorbed
32 to phyllosilicate clay minerals, J. Hydrol., 515, 1–9, doi:10.1016/j.jhydrol.2014.04.029, 2014.

33 O'Neil, J. R. and Truesdell, A. H.: Oxygen isotope fractionation studies of solute-water
34 interactions, in Stable Isotope Geochemistry: A Tribute to Samuel Epstein, vol. 3, edited by H.
35 . Taylor Jr., J. R. O'Neil, and I. R. Kaplan, pp. 17–25, Geochem. Soc, Washington, D. C., USA.,
36 1991.

Orlowski, N., Frede, H.-G., Brüggemann, N. and Breuer, L.: Validation and application of a cryogenic vacuum extraction system for soil and plant water extraction for isotope analysis, J. Sens. Sens. Syst., 2(2), 179–193, doi:10.5194/jsss-2-179-2013, 2013.

Orlowski, N., Breuer, L. and McDonnell, J. J.: Critical issues with cryogenic extraction of soil water for stable isotope analysis, Ecohydrol., 9(1), 1–5, doi:10.1002/eco.1722, 2016.

Orlowski, N., Pratt, D. L. and McDonnell, J. J.: Intercomparison of soil pore water extraction methods for stable isotope analysis, Hydrol. Process., 30(19), 3434–3449, doi:10.1002/hyp.10870, 2016b.

Orlowski, N., Winkler, A., McDonnell, J. J. and Breuer, L.: A simple greenhouse experiment to explore the effect of cryogenic water extraction for tracing plant source water, Ecohydrology, e1967, doi:10.1002/eco.1967, 2018.

Penna, D., Stenni, B., Šanda, M., Wrede, S., Bogaard, T. A., Michelini, M., Fischer, B. M. C., Gobbi, A., Mantese, N., Zuecco, G., Borga, M., Bonazza, M., Sobotková, M., Čejková, B. and Wassenaar, L. I.: Technical Note: Evaluation of between-sample memory effects in the analysis of δ^2H and δ^{18}O of water samples measured by laser spectroscopes, Hydrol. Earth Syst. Sci., 16(10), 3925–3933, doi:10.5194/hess-16-3925-2012, 2012.

Poppe, L. J., Paskevich, V. F., Hathaway, J. C. and Blackwood, D. S.: USGS, U.S. Geological Survey, Coastal and Marine Geology Program, Open-File Report 01-041: A Laboratory Manual for X-Ray Powder Diffraction, http://pubs.usgs.gov/of/2001/of01-041/htmldocs/intro.htm (Accessed 12 October 2016). 2016.

R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria., 2014.

Rozanski, K., Araguás-Araguás, L. and Gonfiantini, R.: Isotopic Patterns in Modern Global Precipitation, in Climate Change in Continental Isotopic Records, edited by P. K. Swart, K. C. Lohmann, J. Mckenzie, and S. Savin, pp. 1–36, American Geophysical Union, Washington, D. C., US., 1993.

Savin, S. M. and Epstein, S.: The oxygen and hydrogen isotope geochemistry of clay minerals, Geochim. Cosmochim. Acta, 34(1), 25–42, doi:10.1016/0016-7037(70)90149-3, 1970.

Schultz, N. M., Griffis, T. J., Lee, X. and Baker, J. M.: Identification and correction of spectral contamination in ^{2}H/^{1}H and ^{18}O/^{16}O measured in leaf, stem, and soil water, Rapid Commun. Mass Spectrom., 25(21), 3360–3368, doi:10.1002/rcm.5236, 2011.

Schuttlefield, J., Cox, D. and Grassian, V.: An investigation of water uptake on clays minerals using ATR-FTIR spectroscopy coupled with quartz crystal microbalance measurements, J. Geophys. Res. Atmospheres, 112(21), 1-14., doi:10.1029/2007JD008973, 2007.

Sprenger, M., Herbstritt, B. and Weiler, M.: Established methods and new opportunities for pore water stable isotope analysis, Hydrol. Process., 29(25), 5174–5192, doi:10.1002/hyp.10643, 2015.

1 Van De Velde, J. H. and Bowen, G. J.: Effects of chemical pretreatments on the hydrogen
2 isotope composition of 2:1 clay minerals, Rapid Commun. Mass Spectrom., 27(10), 1143–
3 1148, doi:10.1002/rcm.6554, 2013.

4 Walker, G. R., Woods, P. H. and Allison, G. B.: Interlaboratory comparison of methods to
5 determine the stable isotope composition of soil water, Chem. Geol., 111(1–4), 297–306,
6 doi:10.1016/0009-2541(94)90096-5, 1994.

7 Wassenaar, L., Ahmad, M., Aggarwal, P., van Duren, M., Pöltenstein, L., Araguas, L. and
8 Kurttas, T.: Worldwide proficiency test for routine analysis of δ^2H and δ^{18}O in water by isotope-
9 ratio mass spectrometry and laser absorption spectroscopy, Rapid Commun. Mass Spectrom.,
10 26(15), 1641–1648, doi:10.1002/rcm.6270, 2012.

11 Wassenaar, L. I., Terzer-Wassmuth, S., Douence, C., Araguas-Araguas, L., Aggarwal, P. K.
12 and Coplen, T. B.: Seeking excellence: An evaluation of 235 international laboratories
13 conducting water isotope analyses by isotope-ratio and laser-absorption spectrometry, Rapid
14 Commun. Mass Spectrom., 32(5), 393–406, doi:10.1002/rcm.8052, 2018.

15 West, A. G., Patrickson, S. J. and Ehleringer, J. R.: Water extraction times for plant and soil
16 materials used in stable isotope analysis, Rapid Commun. Mass Spectrom., 20(8), 1317–1321,
17 doi:10.1002/rcm.2456, 2006.

18 White, w., Arthur and Pichler, E.: Water Sorption Properties of Clay Minerals. (No.
19 208), https://www.ideals.illinois.edu/bitstream/handle/2142/44988/watersorptioncha266whit.
20 pdf?sequence=2, 1959.

21 Zieffler, A. S., Harring, J. R. and Long, J. D.: 12. Unplanned Contrasts, in Comparing Groups:
22 Randomization and Bootstrap Methods Using R, pp. 255–286, John Wiley & Sons, New York,
23 NY, USA., 2012.

1 **Tables**

2 Table 1: Description of the respective extraction systems that participated in the cryogenic

3 inter-laboratory comparison, the applied extraction parameters for extraction approach I and

4 the amount of sample material used for both extraction methods (lab-procedure: I and pre-

5 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

16	Germany	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

1 **Figures**

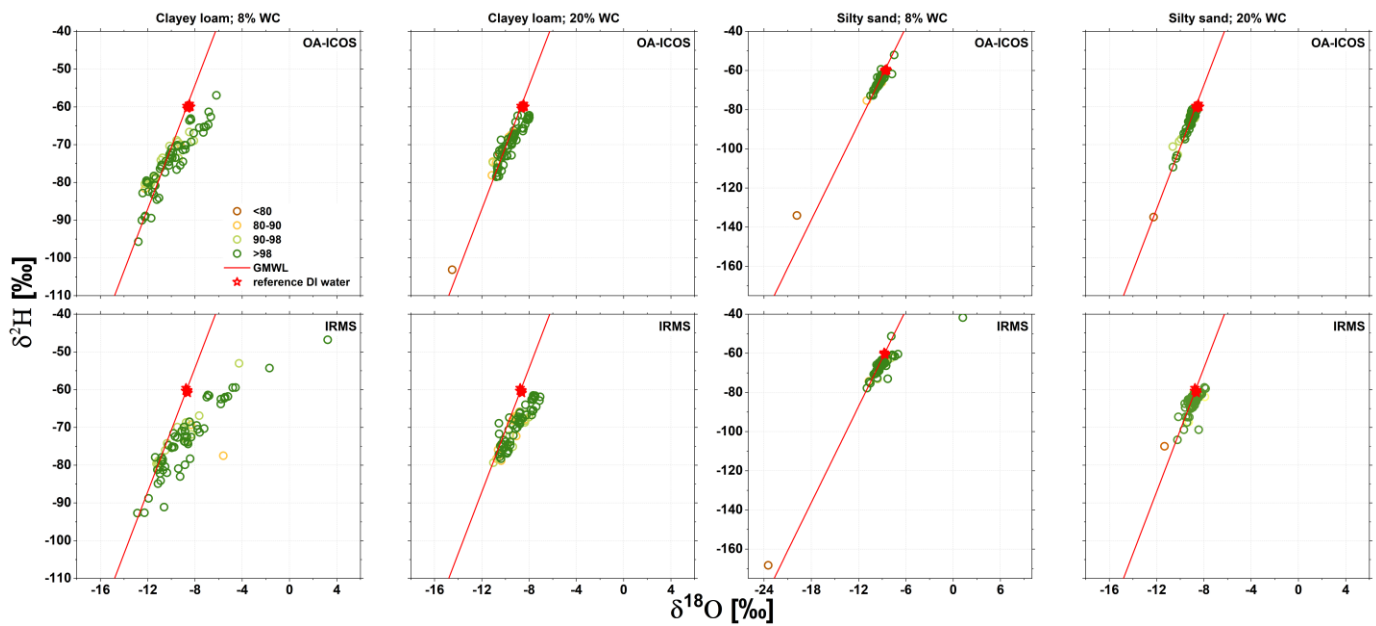
2 Figure 1. Water recovery rates (grouped from <80 to >98%) for both soil types (clayey loam

3 and silty sand), WCs (8% and 20%) and OA-ICOS and IRMS (upper and lower panels,

4 respectively) isotope data in comparison to the spiked reference DI water (red asterisks) shown

5 in dual isotope space. For reference, plots include the Global Meteoric Water Line (GMWL,

6 solid red line). Water recovery rates are shown for those labs that provided the complete set of

7 soil weight data (in % of previously added water).

1   Figure 2. Effect of cryogenic extraction parameters (duration, temperature, and pressure) on

2   δ^2H results of both soil types (clayey loam and silty sand) and WCs (8 and 20%) shown for all

3   labs. The mean reference DI water δ^2H value is included as red dotted line.

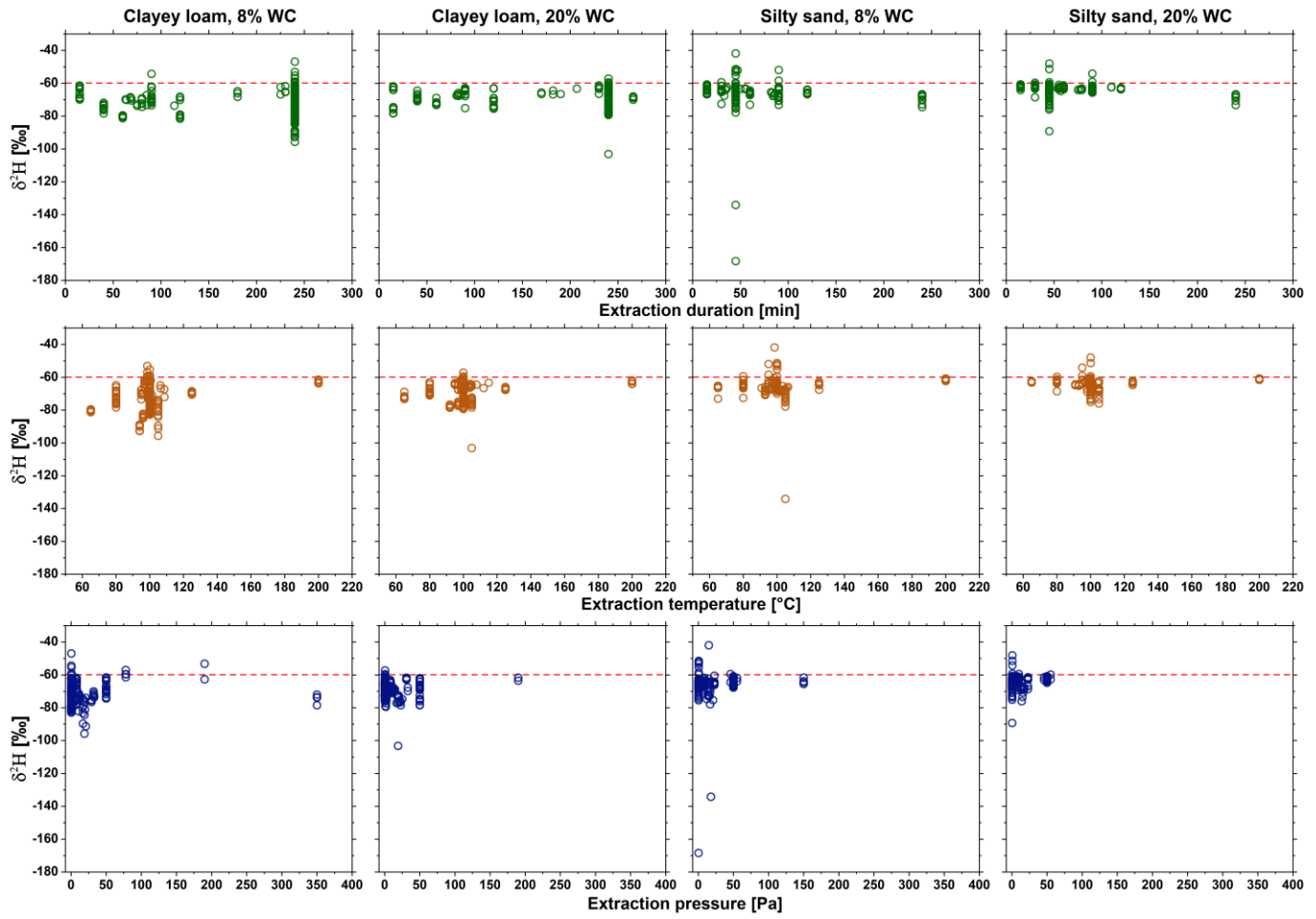



Figure 3. Mean differences from reference DI water for δ^2H OA-ICOS results of water extracts from both extraction methods (lab-procedure: I and pre-defined: II), soil types, and water contents (8 and 20% WC) including TSD of ±2 for δ^2H (Asterisk: -108.4 for δ^2H). Symbols represent the mean of the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

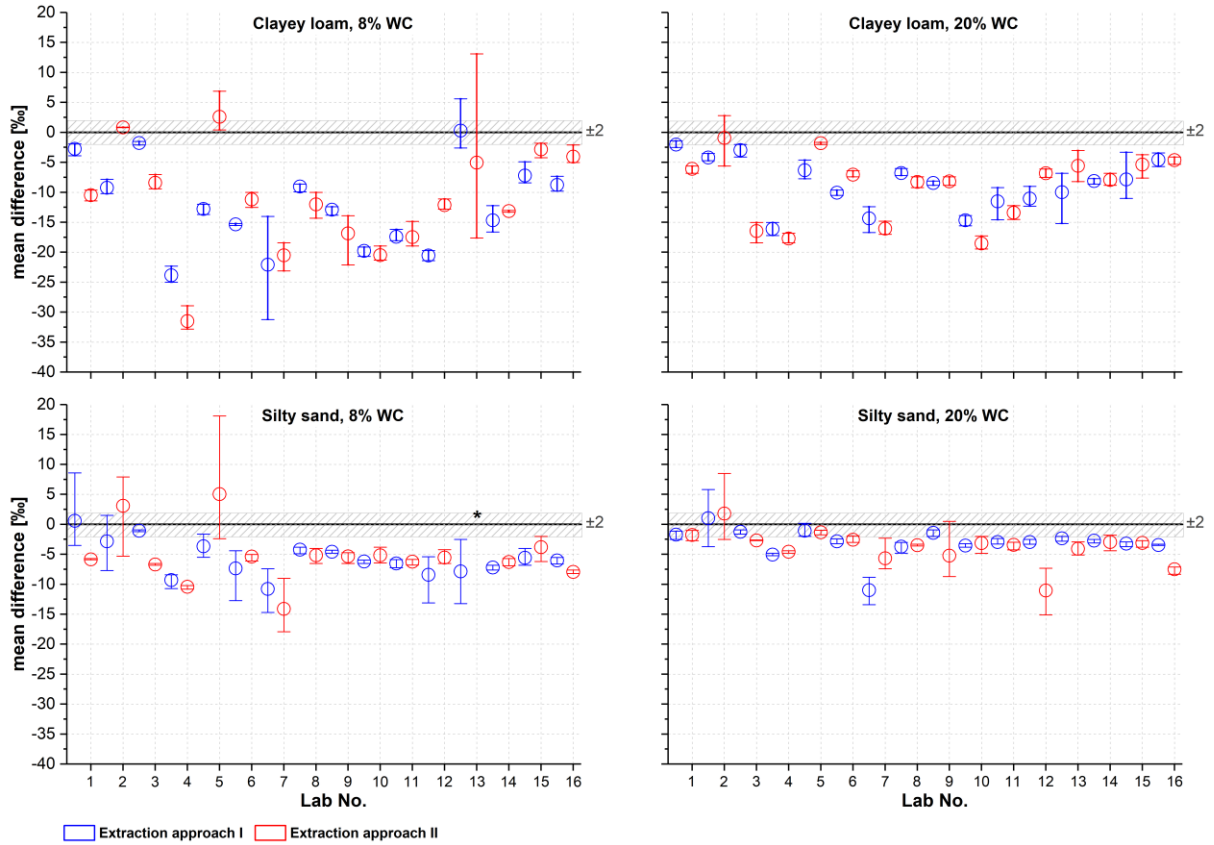
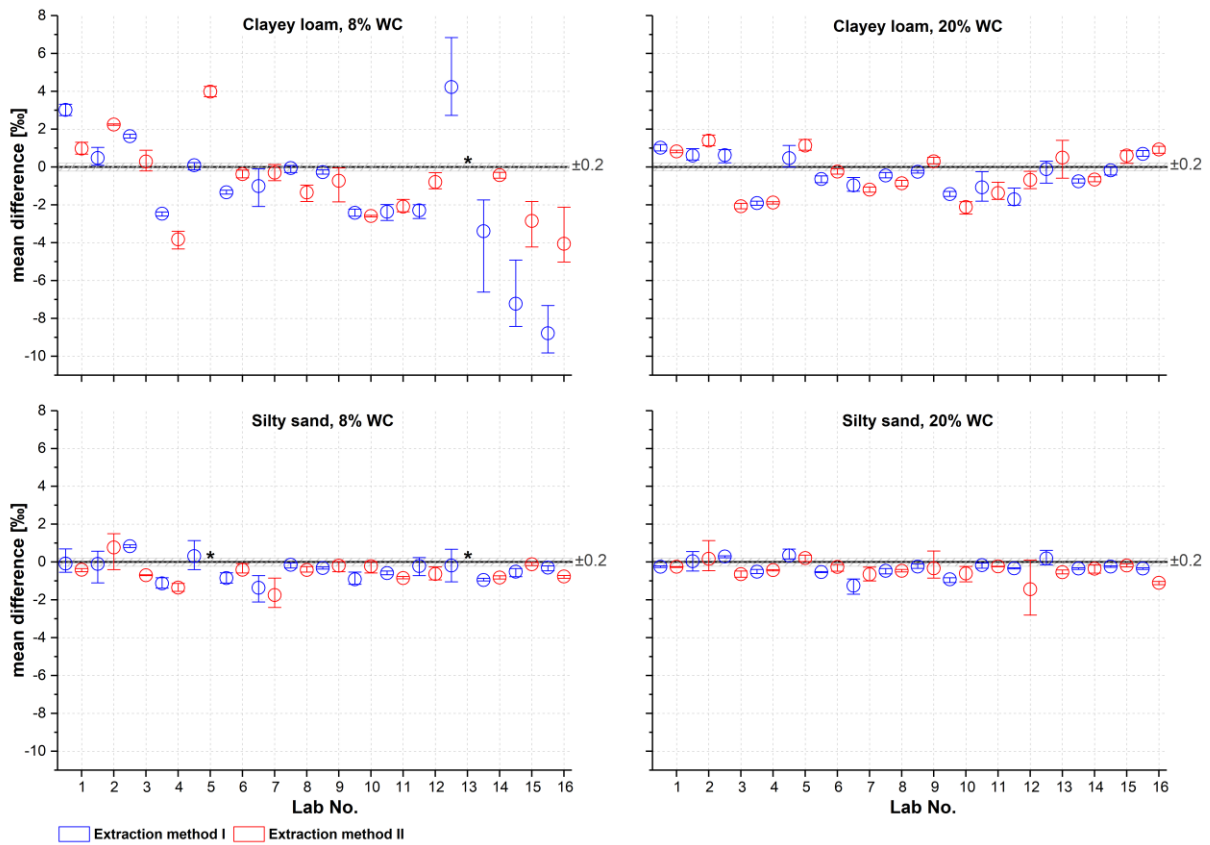



Figure 4. Mean differences from reference DI water for δ^{18}O OA-ICOS results of water extracts from both extraction methods (lab-procedure: I and pre-defined: II), soil types, and water contents (8 and 20% WC) including TSD of ±0.2 for δ^{18}O. Asterisks represent outliers. Symbols represent the mean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

1 Figure 5. Dual isotope plots of clayey loam extracts for 8% and 20% WC in comparison to
2 reference DI water (red asterisks) for OA-ICOS and IRMS data (upper and lower panels,
3 respectively) from the 16 participating labs (different colors represent different labs) and both
4 extraction methods (lab-procedure: I and pre-defined: II). For reference, plots include the
5 Global Meteoric Water Line (GMWL, solid red line) and soil water regression lines for 8% and
6 20% WC (solid green and orange lines, respectively).

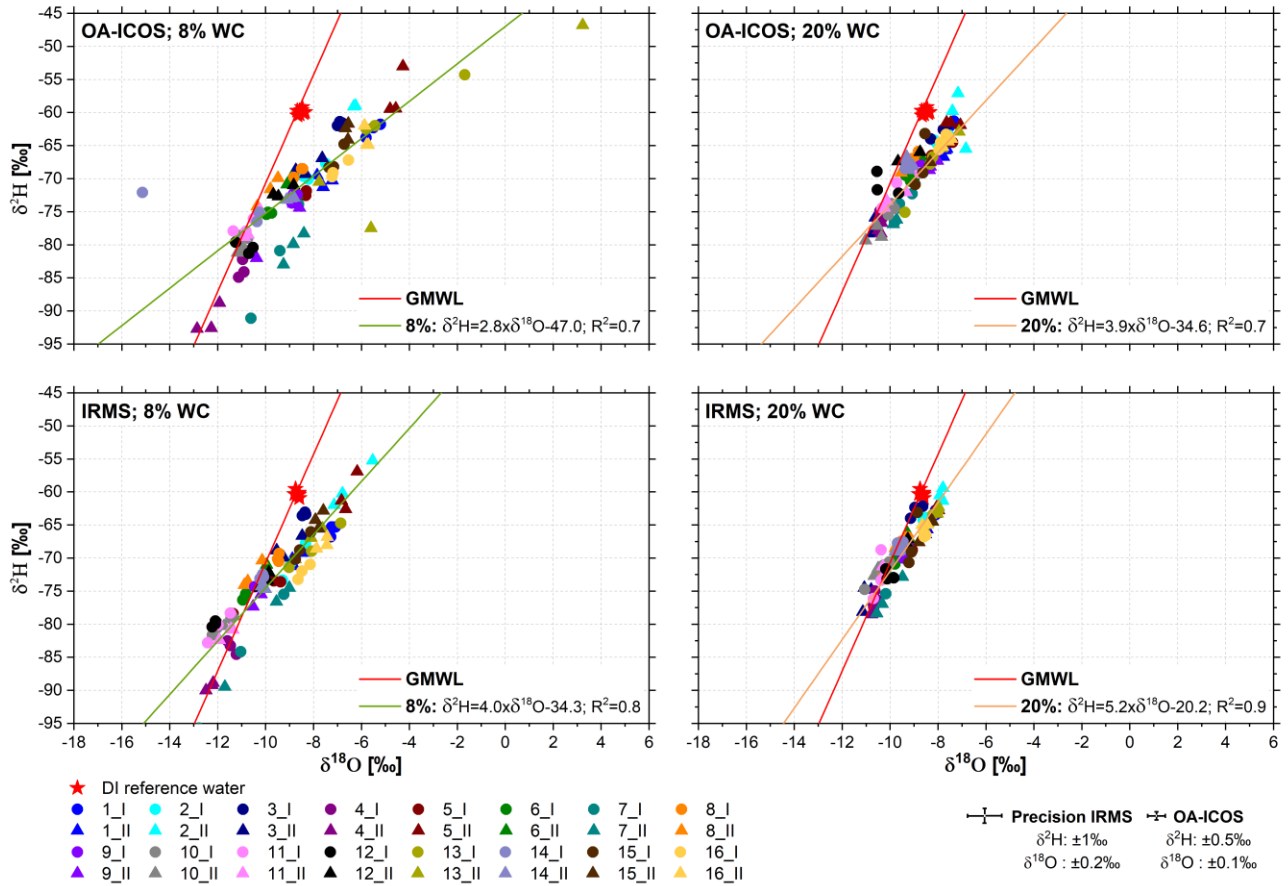
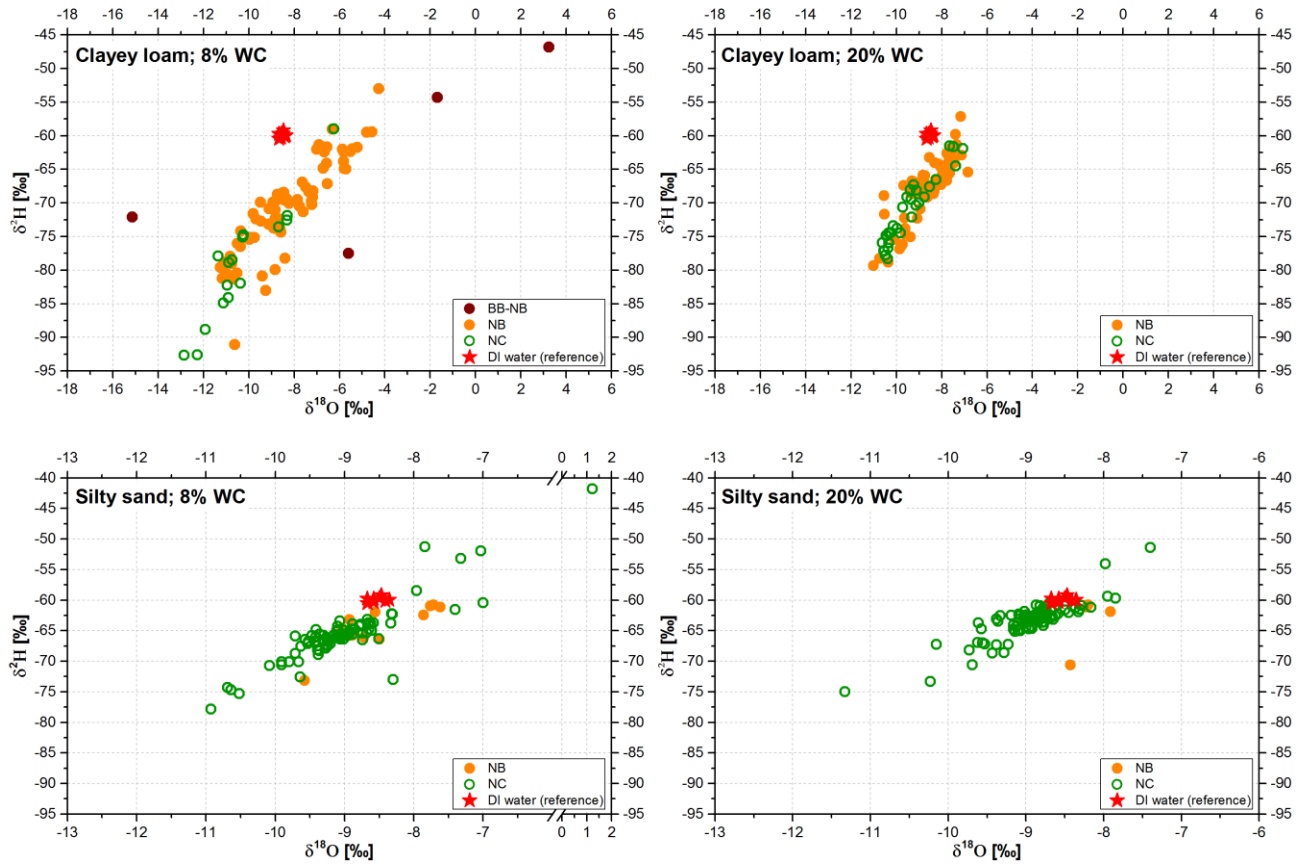



Figure 6. Dual isotope plots of silty sand extracts for 8% and 20% WC in comparison to reference DI water (red asterisks) for OA-ICOS and IRMS data (upper and lower panels, respectively) from the 16 participating labs (different colors represent different labs) and both extraction methods (lab-procedure: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

1 Appendix

2 Appendix 1. Cryogenic system – Questionnaire

4 Inter-laboratory comparison of CWE systems

Contact person

Last Name	First Name

Address

Street	Street No.

City	Postal Code	Country

Phone Number	Email

10 Cryogenic system – Questionnaire
11
12 How many numbers of extraction slots/units does your cryogenic extraction system have?

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

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

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

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

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

24
25 Please provide us a photo of your cryo-line.