



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

3

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

10

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

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

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

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

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

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

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

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

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

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

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

31 [l] Laboratory of Hydrology and Water management, Faculty of Bioscience Engineering, University of
32 Ghent, Ghent, Belgium

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

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

36 [o] INRA UR1138 Biogéochimie des Ecosystèmes Forestiers, INRA Centre de Nancy, Champenoux,
37 France



- 1 [p] Plant Ecology and Ecosystems Research, University of Goettingen, Goettingen, Germany
2 [q] Ecophysiology of Plants, Technical University Munich, Munich, Germany
3 [r] Stable Isotope Research Facility, Paul Scherrer Institute (PSI), Villigen, Switzerland
4 [s] Institute of Groundwater Ecology, German Research Center for Environmental Health, Helmholtz
5 Zentrum München, Neuherberg, Germany; now at: Institute of Hydraulics and Rural Water
6 Management (IHLW), University of Natural and Life Sciences (BOKU) Vienna, Austria
7 [t] Institute of Soil Science, University of Hamburg, Hamburg, Germany
8 *Correspondence to: N. Orlowski, now at: Faculty of Environment and Natural Resources, Chair of
9 Hydrology, Albert-Ludwigs University Freiburg, Freiburg, Germany
10 (Natalie.Orlowski@hydrology.uni-freiburg.de)

11

12 Abstract

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



1 types of cryogenic extraction lab construction varied from manifold systems to single chambers,
2 no clear trends between system construction, applied extraction conditions, and extraction
3 results were found. Rather, differences between isotope results were influenced by interactions
4 between multiple factors (soil type and properties, soil water content, system setup, extraction
5 efficiency, extraction system leaks, and each lab's internal accuracy). Our results question the
6 usefulness of cryogenic extraction as a standard for water extraction since results are not
7 comparable across laboratories. This suggests that defining any sort of standard extraction
8 procedure applicable across laboratories is challenging. Laboratories might have to establish
9 calibration functions for their specific extraction system for each natural soil type, individually.

10

11 **1 Introduction**

12 The interpretation of the stable isotope signatures of water ($\delta^2\text{H}$ and $\delta^{18}\text{O}$) 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; Orłowski 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; Orłowski 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; Orłowski 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; Orłowski 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;



1 Koeniger et al., 2011; Orlowski et al., 2013). Thus, no standard system setup or methodology
2 exists.

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

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

19

20 **2 Methods**

21 **2.1 Experimental design**

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

25

26 [Table 1 near here]

27

28 Before the commencement of the round robin test, participants were asked to fill out a
29 questionnaire (see Appendix 1) to characterize their cryogenic extraction system in terms of
30 numbers of extraction slots or amount of sample material usually introduced into the system
31 (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 (LUF A Speyer,
2 2015) (Table 2) were used for the interlaboratory comparison.

3

4

[Table 2 near here]

5

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): $\delta^2\text{H}$:
17 $-59.8\pm 0.2\text{‰}$ and $\delta^{18}\text{O}$: $-8.5\pm 0.1\text{‰}$, $n=6$; and via Delta VTM Advantage mass spectrometer
18 (Thermo Fisher Scientific, Waltham, MA, US): $\delta^2\text{H}$: $-60.5\pm 0.2\text{‰}$ and $\delta^{18}\text{O}$: $-8.7\pm 0.1\text{‰}$, $n=6$).
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-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.



1

2 **2.2 Sample preparation protocol**

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

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

28

29 **2.3 Cryogenic extraction approaches**

30 Each laboratory was instructed to follow two different extraction approaches: (I) For the first
31 subset of rehydrated soil samples, participants applied the CWE procedure considered routine
32 in their laboratory for the specific soil type and soil water content. (II) With the second subset,



1 CWE under pre-defined conditions for all labs was performed: For silty sand, a 45 min
2 extraction time was used while 240 min was applied to clayey loam samples, both at an
3 extraction temperature of 100°C and a vacuum of 0.3 Pa. These pre-defined extraction
4 parameters were identical for all participating laboratories. Three replicates per soil type and
5 soil water content resulting in 24 samples per extraction procedure (pre-defined and laboratory
6 specific) and isotope analysis method (OA-ICOS and IRMS) were processed (n=48 in total).
7 Pre- and post-oven-dried (105°C for 24 h) soil sample weights were used to determine water
8 recovery rates. All extracted water samples were transferred to 2 mL amber glass vials capped
9 with solid lids (Th. Geyer Inc., Renningen, DE), tightly sealed with Parafilm®, labeled, and
10 shipped to the GIWS for isotope analysis. If the amount of extracted water was not sufficient
11 to entirely fill the 2 mL vial, inserts (0.2 mL) were used (Th. Geyer Inc., Renningen, DE) to
12 minimize sample vial headspace, following standard procedures as outlined by the IAEA
13 (2014).

14

15 **2.4 Isotope analyses**

16 For cross-checking isotope results and ruling out potential lab analytical differences, the
17 isotopic composition of the extracted water samples was analyzed via both OA-ICOS and
18 IRMS. OA-ICOS samples were analyzed on an IWA-45EP Analyzer (Los Gatos Research Inc.,
19 Mountain View, US). The accuracy of OA-ICOS analyses was $\pm 0.5\text{‰}$ for $\delta^2\text{H}$ and $\pm 0.1\text{‰}$ for
20 $\delta^{18}\text{O}$. IRMS samples were analyzed on a Delta V™ Advantage mass spectrometer (Thermo
21 Fisher Scientific, Waltham, MA, US) and an H/Device peripheral using a Cr-reduction method
22 for $\delta^2\text{H}$ analysis (Morrison et al., 2001). For $\delta^{18}\text{O}$ analysis, a GasBench II peripheral was
23 utilized. Using mass spectrometry, a conversion from the water into a light gas suitable for mass
24 spectrometry (H_2 , CO_2 , CO , O_2) is necessary. This conversion step often turns out to limit the
25 achievable precision of IRMS (Brand et al., 2009). In our case, IRMS results are accurate to
26 $\pm 1\text{‰}$ for $\delta^2\text{H}$ and to $\pm 0.2\text{‰}$ for $\delta^{18}\text{O}$, respectively. All isotope ratios are reported in per mil (‰)
27 relative to Vienna Standard Mean Ocean Water (VSMOW) ($\delta^2\text{H}$ or
28 $\delta^{18}\text{O} = (\text{R}_{\text{sample}}/\text{R}_{\text{standard}} - 1) \times 1000\text{‰}$), where R is the isotope ratio of the sample and the known
29 reference (i.e. VSMOW) (Craig, 1961). In-house standards, calibrated against VSMOW2 and
30 SLAP2, were run as samples to allow the results to be reported against VSMOW (Nelson,
31 2000).



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

9

10 **2.5 Statistical evaluation**

11 We used R for statistical analyses (R version 3.3.2; R Core Team, 2014). For quantifying
12 laboratory variances, differences between pre-defined and laboratory specific extraction
13 procedures, effects of soil type and WC, differences between OA-ICOS and IRMS, all data
14 were tested for normality using the Shapiro-Wilk test. Homoscedasticity was tested using either
15 the Levene's test for normally distributed data or the Fligner-Killeen test for non-normally
16 distributed data. Cook's distance was determined in order to identify outliers ($D > 1$). Depending
17 on the type of data (normally distributed and homoscedastic), either Kruskal-Wallis rank sum
18 tests or Analyses of Variances (ANOVAs) were applied and posthoc tests (e.g., Nemenyi-tests)
19 were run to determine which groups were significantly different ($p \leq 0.05$). P-value adjustments
20 via the FDR-method (false discovery rate) were applied to reduce the family-wise-error rate
21 (Zieffler et al., 2012).

22 For graphical comparisons, a target standard deviation (TSD) for acceptable performance was
23 set to $\pm 2\%$ for $\delta^2\text{H}$ and $\pm 0.2\%$ for $\delta^{18}\text{O}$ similar to Orlowski et al. (2016b), which is considered
24 reasonable for hydrologic studies (Wassenaar et al., 2012). The TSD does not account for errors
25 associated with the extraction method itself, weighing errors, and volumetric water additions to
26 the sample, or any standard deviations (1SDs) related to the isotope analysis. Statistically
27 significant ($p \leq 0.05$) linear regressions were added to dual isotope plots as references
28 (evaporation water lines) as well as the Global Meteoric Water Line (GMWL: $\delta^2\text{H} = 8.2 \times \delta^{18}\text{O}$
29 $+ 11.3\%$, as defined by Rozanski et al. (1993)).

30



1

2 **3.2 Laboratory performance with respect to water content and soil type**

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

6 For the 8% WC tests, mean differences for the clayey loam ranged from +13.1 to -32.8‰ for
7 $\delta^2\text{H}$. For the individual lab procedure's extraction approach (I) at 8% WC for the clayey loam,
8 two laboratories (lab 3 and 8) were able to get back to the reference $\delta^2\text{H}$ value based on no
9 statistically significant differences ($p>0.05$) (Fig. 3, upper left plot). For the pre-defined
10 extraction approach (II) at 8% WC, two other labs recovered the $\delta^2\text{H}$ value from the clayey
11 loam (lab 9 and 15).

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

16

17 [Figure 3 near here]

18

19 Mean differences between the silty sand water extraction and the reference $\delta^2\text{H}$ signature were
20 in a smaller range of $\pm 18\%$ than clayey loam extracts from the same treatment (8% WC).

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

25 For silty sand at 20% WC, most laboratories' results even fell close to the range of the TSD of
26 $\pm 2\%$. Mean differences to the reference DI water $\delta^2\text{H}$ signature ranged from +8.5 to -15.1‰
27 (Fig. 3, lower right plot). However, extraction approach I was statistically not successful in
28 recovering the added label ($p<0.05$), but five laboratories (6, 9, 10, 14, and 15) showed no
29 significant differences to the reference DI water when applying extraction approach II to the
30 silty sand at 20% WC.

31 Laboratories performed better for $\delta^{18}\text{O}$ signature recovery, especially with extraction approach
32 I. For both clayey loam WC treatments, labs 13 and 15 were the most successful. Again, mean



1 differences to the reference DI water were larger for the 8% WC than for the 20% WC (Fig. 4,
2 upper plots). However, for the clayey loam at 20% WC with the pre-defined approach (II) only
3 lab 13 and 14 did not show statistically significant differences to the added $\delta^{18}\text{O}$ signature
4 (Figure 4, upper right plot) ($p>0.05$).

5

6

[Figure 4 near here]

7

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

11

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

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

23

24 **3.3 Differences between OA-ICOS- and IRMS-based measurements**

25 Figures 5 (clayey loam) and 6 (silty sand) illustrate data variability for each laboratory and WC
26 with respect to the labeled reference DI water added to each soil type in dual isotope space.
27 Significant differences were observed between OA-ICOS and IRMS isotope data sets ($p\leq 0.05$).
28 The clay soil isotope data at 8% WC showed the greatest differences between OA-ICOS and
29 IRMS measurements (mean differences of 1.3 and 1.2 for $\delta^2\text{H}$ and $\delta^{18}\text{O}$, respectively). Smallest
30 differences between isotope analyzers were observed between both WC treatments of the silty
31 sand (Fig. 6). The data sets with the lowest SD for both isotopes across labs and extraction
32 approaches were the silty sand samples at 20% WC measured via OA-ICOS and IRMS (SD of



1 ± 3.1 for $\delta^2\text{H}$ measured via OA-ICOS and ± 4.2 for IRMS, respectively). However, those data
2 sets still did not reach the TSD of $\pm 2\%$ for $\delta^2\text{H}$ and $\pm 0.2\%$ for $\delta^{18}\text{O}$.

3

4

[Figure 5 near here]

5

6 For comparison, apart from evaporation lines, the GMWL is also given in each subplot.
7 Interestingly, isotope data across laboratories plot on slopes lower than the GMWL. For both
8 soil types, evaporation lines of the IRMS measurements showed better correlations (for the silty
9 sand $R^2=0.8$ and 0.9 for 8% and 20% WC, respectively) than those of OA-ICOS measurements
10 ($R^2=0.7$ for 8% and 20% WC) (Fig. 6). Silty sand's soil evaporation water lines showed greater
11 slopes ($5.4\text{--}7.2$ across both WCs and isotope analysis) than clayey loam's soil evaporation
12 water lines ($2.8\text{--}5.2$ across both WCs and isotope analysis) (Figures 5 and 6). The clayey loam
13 evaporation lines for the higher WC also showed greater slopes than those of the lower WC
14 (Fig. 5). Isotopic fractionation due to evaporation leads to a stronger kinetic effect for ^{18}O
15 compared to ^2H , resulting in evaporative enrichment of the water along an evaporation water
16 line (e.g., soil evaporation water line) with a lower slope relative to the original water
17 (Gonfiantini, 1986) –in our case, the reference DI water.

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

21

22

[Figure 6 near here]

23

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

31 Examination of the differences between OA-ICOS and IRMS data, prompted to test the OA-
32 ICOS data for spectral interferences. Figure 7 shows that for the clayey loam soil, differences



1 between OA-ICOS and IRMS data might be due to co-extracted alcoholic compounds, which
2 caused erroneous OA-ICOS data.

3

4

[Figure 7 near here]

5

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

14

15 **4 Discussion**

16 **4.1 Why are the cryogenic extraction results different across the participating laboratories?**

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

22 Each extraction system setups were different. Therefore, it was difficult to give any
23 recommendation with regard to a high-performance and accurate extraction system that would
24 lead to overall successful extractions. As a quality control, we checked water recovery rates,
25 which were in some cases even higher than 100% (Fig. 1). This could be attributed either to
26 leaky vacuum systems (which might allow atmospheric water vapor to enter the system) or to
27 a remoistening of the oven-dried soil samples before water extraction. Remoistening of oven-
28 dried soil samples might be a general problem of such spiking experiments. In our case, sample
29 preparation was not performed under an inert gas flow and, unfortunately, data on temperature
30 and relative humidity conditions under which sample preparation took place are unavailable
31 from the respective labs. Ambient water vapor isotopic composition measurements would have



1 also been a relevant additional information. Contamination could also occur when an extraction
2 system is not dried or cleaned after each extraction run, leaving moisture and/or soil material
3 behind which would affect the next sample's results. Other measurement uncertainties during
4 the extraction protocol could arise from weighing errors (scale calibration and precision), the
5 accuracy of the volume of water additions to the soil samples, transfer of the samples, loss of
6 water vapor during evacuation of the extraction system, unsteady heating temperatures,
7 condensation of water vapor in the extraction system, and a lack of precision of analytical and
8 laboratory equipment.

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

15 As an additional performance test, laboratories were asked to perform water-to-water
16 extractions to show their ability to recover water of known isotopic composition prior to soil-
17 based tests. For example, some laboratories, like lab 2, showed a high accuracy for these water-
18 to-water extractions of $\pm 0.4\%$ for $\delta^2\text{H}$ and $\pm 0.1\%$ for $\delta^{18}\text{O}$ ($n=119$) as well as lab 16. They
19 performed extraction tests with tap water, which resulted in no significant differences between
20 the initial, untreated ($-56.7\% \pm 0.4$ for $\delta^2\text{H}$ and $-9.3\% \pm 0.1$ for $\delta^{18}\text{O}$) and extracted tap water
21 ($-57.5\% \pm 0.6$ for $\delta^2\text{H}$ and $-9.4\% \pm 0.1$ for $\delta^{18}\text{O}$). These examples show that these labs among
22 others were able to reach the TSD with simple water-to-water extractions, but with soils, they
23 were unsuccessful. This indicates that differences between the reference DI water and water
24 spiked and extracted from soils are likely caused by interactions with soil particles.

25 Given our findings, we now question the standard quality controls (e.g., water recovery rate
26 calculations and water-to-water extractions). Quality controls with spiked soil samples may be
27 a more effective way to demonstrate lab's internal accuracy. However, such spiking
28 experiments as performed in our study come along with other issues as recently outlined by Gaj
29 et al. (2017b) and Sprenger et al. (2015). Gaj et al. (2017a) applied the Rayleigh equation (using
30 stable isotope signatures) to calculate how much water was cryogenically extracted from pure
31 clay minerals. They found that for samples from which water has been extracted to 100%



1 (determined gravimetrically), the Rayleigh equation showed that only 72% of water was
2 extracted at a temperature of 105°C. When using an extraction temperature of 205°C, the
3 Rayleigh-estimated amount of water extracted was close to 90%, but still not 100%. This result
4 clearly shows that despite the gravimetric quality control suggesting that all water has been
5 extracted, isotopic differences may still exist.

6 Overall, laboratories 9 (for $\delta^2\text{H}$) and 13 (for $\delta^{18}\text{O}$) were the most successful in getting back to
7 the DI reference water over all soil types and WCs. For the lab's in-house procedure, laboratory
8 9 extracted both soils for 90 min at 95°C and 0.8 Pa. Their reported water extraction efficiency
9 was 99–100%. Glass tubes were used as extraction containers and a water bath as heating
10 element. Laboratory 13 used different extraction parameters, which also varied slightly from
11 sample to sample: for the clayey loam at 8% WC, extractions were conducted for 75–114 min
12 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
13 as in-house extraction parameters. For the silty sand at 8% WC, their extraction time was 15 min
14 at 100°C and 7.3–13.3 Pa. For the 20% WC, they extracted for 30 min at 100°C and 6.7–
15 10.7 Pa. Lab 13 further specified that their extraction times were dictated by a decline in the
16 pressure level indicating that no more water was evaporating from the respective sample.
17 Extraction efficiencies for lab 13 varied between 93–127%. Glass tubes were used as extraction
18 containers along with a sensor-regulated tube-shaped heating element. This example shows that
19 even for the relatively successful laboratories, extraction parameters did not seem to play a
20 major role for achieving the reference DI water isotopic signature.

21

22 **4.2 How do soil type and water content affect the results?**

23 The adsorbed and interlayer water occurring in clayey soils can complicate the interpretation
24 of obtained isotope data. Clay-water sorption capacity is well known (Schuttlefield et al., 2007;
25 White and Pichler, 1959). White and Pichler (1959) found early on that montmorillonite adsorbs
26 more water than kaolinite, illite, and chlorite, while chlorites and illites have similar water-
27 sorption properties. The amount of water absorbed/adsorbed by clay minerals ranges from 800-
28 500% for Na-montmorillonite (Kaufhold and Dohrmann, 2008; White and Pichler, 1959) to as
29 low as 60% of the initial dry weight for biotite (White and Pichler, 1959). The clayey loam in
30 our study was a vermiculite-rich (43 relative %) 2:1 clay type, while the silty sand had a
31 negligible clay-fraction (2.6%) where illite (2:1 clay type) occurred with 28 relative % (Table
32 2).



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

8 Savin and Epstein (1970) also observed that atmospheric vapor exchanged isotopically with
9 interlayer water (almost completely) and Aggarwal et al. (2004) showed that this can occur
10 within hours. This demonstrated that the isotopic composition of clay interlayer and adsorbed
11 water can reflect the isotopic composition of atmospheric water vapor at the storage location.
12 However, once the soil has been heated under vacuum and the interlayer water removed, the
13 remaining water showed no evidence of isotopic exchange. Again, it should be stressed here
14 that for our intercomparison soil samples were oven-dried twice (before and after shipment)
15 prior to any rewetting and labs were advised to store the dried samples in a desiccation chamber
16 until use. However, oven-drying was performed at an intermediate temperature (105°C for 48h)
17 and not under vacuum as per Savin and Epstein (1970) and different indoor laboratory 'climatic
18 conditions' at the participating laboratories were observed. Thus, it might be possible that not
19 all of the clay interlayer and adsorbed water was removed or made isotopically non-
20 exchangeable, and that non-equilibrium isotopic fractionation occurring at different
21 temperatures during heating might be responsible for some of the differences we observed.
22 Thus, sample preparation might have played its role, when it comes to discrepancies in lab's
23 results, especially those at low water contents. At these low water contents, the available water
24 fraction is small and exchange with interlayer and adsorbed water would be proportionally
25 higher. In hindsight, repeating this work with soils dried under vacuum and at higher
26 temperatures (i.e., 300 °C following Savin and Epstein (1970)) may help to clarify and to isolate
27 the effect of remaining water in clay minerals. However, so far, regular oven-drying of soils is
28 standard practice (Koeniger et al., 2011) for such rewetting experiments in the literature.

29 We also observed water content effects on the recovered isotope data as per Meißner et al.
30 (2014). Isotope results across labs were closer to the added reference water isotopic composition
31 at higher WCs. However, this isotope effect cannot be considered independent from other soil
32 property effects such as clay mineral water interactions or effects caused by cation exchange



1 capacity (CEC). Oerter et al. (2014) demonstrated that isotope effects due to soil type are more
2 common in soils with high cation exchange capacity (CEC) at low WCs. This can be further
3 exacerbated by the cations present in the soil. Those soils with high ionic potential (e.g., Ca^{2+}
4 and Mg^{2+}) can create large amounts of structured water surrounding them (hydrated radii)
5 compared to the bulk water in the system. From an oxygen isotope perspective, O'Neil and
6 Truesdell (1991) showed that those cations are capable of causing fractionation between bound
7 and bulk soil water. Moreover, soils higher in potassium ions may have a greater effect on
8 hydrogen isotopes, while sodium soils demonstrate non-fractionating effects (Oerter et al.,
9 2014). These cation fractionation effects for montmorillic soils, in particular, can result in a
10 depletion of up to 1.55‰ in dry soils and 0.49‰ for $\delta^{18}\text{O}$ for wet soils. In our study, chemical
11 and salinity effects –which occur due to the fractionation of water molecules into hydration
12 spheres around fully solvated cations compared to the pure water used to make the solutions –
13 can be ignored for the silty sand due to a low CEC of $4.1 \text{ cmol}(+) \text{ kg}^{-1}$. The high CEC
14 ($30.6 \text{ cmol}(+) \text{ kg}^{-1}$) of the clayey loam soil may have caused some of the detrimental effects
15 seen across laboratories. This is especially the case for low WCs due to ion hydration effects
16 among the cations present (Table 2).

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

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

24

25 **4.3 Differences between OA-ICOS- and IRMS-based measurements**

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

28 Others have found differences in isotope results obtained from OA-ICOS and IRMS (Martín-
29 Gómez et al., 2015; Wassenaar et al., 2012). In a recently performed test, 235 international
30 laboratories conducting water isotope analyses by OA-ICOS and IRMS were evaluated.
31 Wassenaar et al. (2018) could show that inaccuracy or imprecise performance stemmed mainly
32 from skill- and knowledge-based errors including: calculation mistakes, inappropriate or



1 compromised laboratory calibration standards, poorly performing instrumentation, lack of
2 vigilance to contamination, or inattention to unreasonable isotopic outcomes. For the analysis
3 of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ via OA-ICOS, Penna et al. (2012) showed that between-sample memory effects
4 can be an additional problem. Memory effect ranged from 14% and 9% for $\delta^{18}\text{O}$ and $\delta^2\text{H}$
5 measurements, respectively, but declined to 0.1% and 0.3% when the first ten injections of each
6 sample were discarded.

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

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

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

28 Nevertheless, the less expensive, rapid option of the OA-ICOS is still a viable alternative for
29 routine isotope analyses if no organic contamination issues are found and six or more injections
30 are performed and the first two or more are discarded (Penna et al., 2012). If organics are
31 present, proper correction schemes as per Martín-Gómez et al. (2015) need to be applied,
32 especially when OA-ICOS data is used in ecohydrological studies. However, so far, correction



1 procedures only account for contamination caused by methanol or ethanol but plant and soil
2 water extracts can contain a variety of different contaminants. Our work showed that the silty
3 sand soil water extracts were mainly free from organic contamination (Fig. 7). Still, data post-
4 processing is highly recommended to detect issues occurring from co-extracted alcoholic
5 compounds.

6

7 **4.4 Take home messages about cryogenic water extraction**

8 Our lab intercomparison did not find significant correlations between extraction condition
9 parameters such as temperature, time, and applied vacuum, and the obtained isotope results
10 (Fig. 2). Others have shown that extraction time and temperature have significant effects on the
11 CWE isotope results (Goebel and Lascano, 2012; Koeniger et al., 2011; Orłowski et al., 2013,
12 2016a; West et al., 2006). Gaj et al. (2017b) showed clear relationships between temperature
13 and the release of water from interlayer cations and organics during CWE, which affected
14 isotope results. They suggested using temperatures between 200°C and 300°C for clay water
15 extractions. However, higher temperatures could cause a release of water by oxidation of
16 organics and dihydroxylation of hydroxide-containing minerals, and this water might not be an
17 ecohydrologically active part in the water cycle. Moreover, the co-extraction of organics could
18 become more important at harsher extraction conditions leading to spectral interferences when
19 OA-ICOS is used. Our interlab comparison was not able to provide any recommendations with
20 regard to higher temperatures or longer extraction times leading to possibly better extraction
21 results. Little is known about how the applied extraction pressure affects the CWE isotope
22 results. But one thing is clear: that CWE is a ‘brute force technique’ (Orłowski et al., 2016a) in
23 the sense that it is not able to distinguish between waters held at different soil tensions being of
24 different importance for the ecohydrological water cycle. New instrumentation to sample
25 discretely along the moisture release curve is desperately needed (McDonnell, 2014).

26 We found significant differences between extraction approach I (lab “in-house” procedure) and
27 II (pre-defined extraction parameters). Both approaches showed significant differences to the
28 added reference water for the OA-ICOS results, but in different ways. For example, for $\delta^2\text{H}$
29 signature recovery from silty sand, extraction approach II worked better. The same was true for
30 $\delta^{18}\text{O}$ signature recovery for both WC treatments. However, for other settings, it was difficult to
31 identify the ideal extraction approach that got closer to the reference DI water isotopic



1 composition. We found no clear tendency for which approach should be applied, thus at present,
2 and much to our dismay, we cannot define any standard protocol for CWE.

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

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

18

19 **5 Conclusions**

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

30 Although the applied extraction system setups were different (e.g., size of extraction container,
31 heating unit), we could not show a major impact of the system's design on the isotope results,



1 as laboratories were successful for the one soil type and water content but failed for the other.
2 However, internal reproducibility of isotope results for the replicates of the same soil type at a
3 given WC was given for most of the labs. Nevertheless, different results were obtained for $\delta^{18}\text{O}$
4 and $\delta^2\text{H}$.
5 Our intercomparison work showed that defining any sort of standard extraction procedure for
6 CWEs across laboratories is challenging. Our results question the usefulness of this method as
7 a standard for water extraction since results are not intercomparable across laboratories. A
8 possible option might be that CWE labs establish system-specific calibration functions for each
9 natural soil type, individually, to correct for the given offset to a set of reference soils.
10 Finally, we note that while CWEs for soils are problematic for reasons discussed in this paper,
11 no work yet has seen any effects for plant water extractions apart from spectral interferences
12 when using OA-ICOS. However, such inter-laboratory and technique intercomparison should
13 be addressed for plants in the future to account for possible effects. New continuous, in-situ
14 measurements of soil and plant water isotopic composition might overcome isotope
15 fractionation issues we observed with CWE.
16



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.

8



1 References

- 2 Aggarwal, P. K., Dillon, M., A. and Tanweer, A.: Isotope fractionation at the soil-atmosphere interface
3 and the ^{18}O budget of atmospheric oxygen, *Geophys. Res. Lett.*, 31(14), 1–4,
4 doi:10.1029/2004GL019945, 2004.
- 5 Araguás-Araguás, L., Rozanski, K., Gonfiantini, R. and Louvat, D.: Isotope effects accompanying
6 vacuum extraction of soil water for stable isotope analyses, *J. Hydrol.*, 168(1–4), 159–171,
7 doi:10.1016/0022-1694(94)02636-P, 1995.
- 8 Brand, W. A., Geilmann, H., Crosson, E. R. and Rella, C. W.: Cavity ring-down spectroscopy versus
9 high-temperature conversion isotope ratio mass spectrometry; a case study on $\delta^2\text{H}$ and $\delta^{18}\text{O}$ of pure
10 water samples and alcohol/water mixtures, *Rapid Commun. Mass Spectrom.*, 23(12), 1879–1884,
11 doi:10.1002/rcm.4083, 2009.
- 12 Craig, H.: Standard for Reporting Concentrations of Deuterium and Oxygen-18 in Natural Waters,
13 *Science*, 133(3467), 1833–1834, doi:10.1126/science.133.3467.1833, 1961.
- 14 Gaj, M., Kaufhold, S., Koeniger, P., Beyer, M., Weiler, M. and Himmelsbach, T.: Mineral mediated
15 isotope fractionation of soil water, *Rapid Commun. Mass Spectrom.*, 31(3), 269–280,
16 doi:10.1002/rcm.7787, 2017a.
- 17 Gaj, M., Kaufhold, S. and McDonnell, J. J.: Potential limitation of cryogenic vacuum extractions and
18 spiked experiments, *Rapid Commun. Mass Spectrom.*, doi:10.1002/rcm.7850, 2017b.
- 19 Goebel, T. S. and Lascano, R. J.: System for high throughput water extraction from soil material for
20 stable isotope analysis of water, *J. Anal. Sci. Methods Instrum.*, 02(04), 203–207,
21 doi:10.4236/jasmi.2012.24031, 2012.
- 22 Gonfiantini, R.: Environmental isotopes in lake studies, in *Handbook of environmental isotope
23 geochemistry: The terrestrial environment*, B, edited by P. Fritz and J. C. Fontes, pp. 113–168,
24 Elsevier, New York, USA., 1986.
- 25 Grim, R. and Bradley, W.: Investigation of the Effect of Heat on the Clay Minerals Illite and
26 Montmorillonite, *J. Am. Ceram. Soc.*, 23(8), 242–248., doi:10.1111/j.1151-2916.1940.tb14263.x,
27 1940.
- 28 IAEA (International Atomic Energy Agency): IAEA/GNIP precipitation sampling guide, [http://www-
29 naweb.iaea.org/napc/ih/documents/other/gnip_manual_v2.02_en_hq.pdf](http://www-naweb.iaea.org/napc/ih/documents/other/gnip_manual_v2.02_en_hq.pdf) (Accessed 15 June 2014),
30 2014.
- 31 Ingraham, N. L. and Shadel, C.: A comparison of the toluene distillation and vacuum/heat methods for
32 extracting soil water for stable isotopic analysis, *J. Hydrol.*, 140(1–4), 371–387, doi:10.1016/0022-
33 1694(92)90249-U, 1992.
- 34 Kaufhold, S. and Dohrmann, R.: Comparison of the traditional Enslin-Neff method and the modified
35 dieng method for measuring water-uptake capacity, *Clays Clay Miner.*, 56(6), 686–692.,
36 doi:10.1346/CCMN.2008.0560609, 2008.
- 37 Koeniger, P., Marshall, J. D., Link, T. and Mulch, A.: An inexpensive, fast, and reliable method for
38 vacuum extraction of soil and plant water for stable isotope analyses by mass spectrometry, *Rapid
39 Commun. Mass Spectrom.*, 25(20), 3041–3048, doi:10.1002/rcm.5198, 2011.
- 40 LUFASpeyer: German State Research Institute for Agriculture, Speyer, DE, [http://www.lufa-
41 speyer.de/index.php/dienstleistungen/standardboeden/8-dienstleistungen/artikel/57-standard-soils](http://www.lufaspeyer.de/index.php/dienstleistungen/standardboeden/8-dienstleistungen/artikel/57-standard-soils)
42 (Accessed 15 April 2015), 2015.
- 43 Martín-Gómez, P., Barbeta, A., Voltas, J., Peñuelas, J., Dennis, K., Palacio, S., Dawson, T. E. and Ferrio,
44 J. P.: Isotope-ratio infrared spectroscopy: a reliable tool for the investigation of plant-water sources?,
45 *New Phytol.*, 1–14, doi:10.1111/nph.13376, 2015.
- 46 McDonnell, J. J.: The two water worlds hypothesis: ecohydrological separation of water between
47 streams and trees?, *Wiley Interdiscip. Rev. Water*, 1(4), 323–329, doi:10.1002/wat2.1027, 2014.
- 48 Meißner, M., Köhler, M., Schwendenmann, L., Hölscher, D. and Dyckmans, J.: Soil water uptake by
49 trees using water stable isotopes ($\delta^2\text{H}$ and $\delta^{18}\text{O}$)—a method test regarding soil moisture, texture and
50 carbonate, *Plant Soil*, 376(1–2), 327–335, doi:10.1007/s11104-013-1970-z, 2014.



- 1 Morrison, J., Brockwell, T., Merren, T., Fourel, F. and Phillips, A. M.: On-line high-precision stable
2 hydrogen isotopic analyses on nanoliter water samples, *Anal. Chem.*, 73(15), 3570–3575, 2001.
- 3 Nelson, S. T.: A simple, practical methodology for routine VSMOW/SLAP normalization of water
4 samples analyzed by continuous flow methods, *Rapid Commun. Mass Spectrom.*, 14(12), 1044–
5 1046, doi:10.1002/1097-0231(20000630)14:12<1044::AID-RCM987>3.0.CO;2-3, 2000.
- 6 Oerter, E., Finstad, K., Schaefer, J., Goldsmith, G. R., Dawson, T. and Amundson, R.: Oxygen isotope
7 fractionation effects in soil water via interaction with cations (Mg, Ca, K, Na) adsorbed to
8 phyllosilicate clay minerals, *J. Hydrol.*, 515, 1–9, doi:10.1016/j.jhydrol.2014.04.029, 2014.
- 9 O’Neil, J. R. and Truesdell, A. H.: Oxygen isotope fractionation studies of solute-water interactions, in
10 *Stable Isotope Geochemistry: A Tribute to Samuel Epstein*, vol. 3, edited by H. . Taylor Jr., J. R.
11 O’Neil, and I. R. Kaplan, pp. 17–25, *Geochem. Soc. Washington, D. C., USA.*, 1991.
- 12 Orłowski, N., Frede, H.-G., Brüggemann, N. and Breuer, L.: Validation and application of a cryogenic
13 vacuum extraction system for soil and plant water extraction for isotope analysis, *J. Sens. Sens. Syst.*,
14 2(2), 179–193, doi:10.5194/jsss-2-179-2013, 2013.
- 15 Orłowski, N., Breuer, L. and McDonnell, J. J.: Critical issues with cryogenic extraction of soil water for
16 stable isotope analysis, *Ecohydrol.*, 9(1), 1–5, doi:10.1002/eco.1722, 2016a.
- 17 Orłowski, N., Pratt, D. L. and McDonnell, J. J.: Intercomparison of soil pore water extraction methods
18 for stable isotope analysis, *Hydrol. Process.*, 30(19), 3434–3449, doi:10.1002/hyp.10870, 2016b.
- 19 Penna, D., Stenni, B., Šanda, M., Wrede, S., Bogaard, T. A., Michelini, M., Fischer, B. M. C., Gobbi,
20 A., Mantese, N., Zuecco, G., Borga, M., Bonazza, M., Sobotková, M., Čejková, B. and Wassenaar,
21 L. I.: Technical Note: Evaluation of between-sample memory effects in the analysis of $\delta^2\text{H}$ and $\delta^{18}\text{O}$
22 of water samples measured by laser spectrometers, *Hydrol. Earth Syst. Sci.*, 16(10), 3925–3933,
23 doi:10.5194/hess-16-3925-2012, 2012.
- 24 Poppe, L. J., Paskевич, V. F., Hathaway, J. C. and Blackwood, D. S.: USGS, U.S. Geological Survey,
25 Coastal and Marine Geology Program, Open-File Report 01-041: A Laboratory Manual for X-Ray
26 Powder Diffraction, <http://pubs.usgs.gov/of/2001/of01-041/htmldocs/intro.htm> (Accessed 12
27 October 2016). 2016.
- 28 R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical
29 Computing, Vienna, Austria., 2014.
- 30 Rozanski, K., Araguás-Araguás, L. and Gonfiantini, R.: Isotopic Patterns in Modern Global
31 Precipitation, in *Climate Change in Continental Isotopic Records*, edited by P. K. Swart, K. C.
32 Lohmann, J. Mckenzie, and S. Savin, pp. 1–36, *American Geophysical Union, Washington, D. C.,*
33 *US.*, 1993.
- 34 Savin, S. M. and Epstein, S.: The oxygen and hydrogen isotope geochemistry of clay minerals, *Geochim.*
35 *Cosmochim. Acta.*, 34(1), 25–42, doi:10.1016/0016-7037(70)90149-3, 1970.
- 36 Schultz, N. M., Griffis, T. J., Lee, X. and Baker, J. M.: Identification and correction of spectral
37 contamination in $^2\text{H}/^1\text{H}$ and $^{18}\text{O}/^{16}\text{O}$ measured in leaf, stem, and soil water, *Rapid Commun. Mass*
38 *Spectrom.*, 25(21), 3360–3368, doi:10.1002/rcm.5236, 2011.
- 39 Schuttlefield, J., Cox, D. and Grassian, V.: An investigation of water uptake on clays minerals using
40 ATR-FTIR spectroscopy coupled with quartz crystal microbalance measurements, *J. Geophys. Res.*
41 *Atmospheres*, 112(21), 1–14., doi:10.1029/2007JD008973, 2007.
- 42 Sprenger, M., Herbstritt, B. and Weiler, M.: Established methods and new opportunities for pore water
43 stable isotope analysis, *Hydrol. Process.*, 29(25), 5174–5192, doi:10.1002/hyp.10643, 2015.
- 44 Van De Velde, J. H. and Bowen, G. J.: Effects of chemical pretreatments on the hydrogen isotope
45 composition of 2:1 clay minerals, *Rapid Commun. Mass Spectrom.*, 27(10), 1143–1148,
46 doi:10.1002/rcm.6554, 2013.
- 47 Walker, G. R., Woods, P. H. and Allison, G. B.: Interlaboratory comparison of methods to determine
48 the stable isotope composition of soil water, *Chem. Geol.*, 111(1–4), 297–306, doi:10.1016/0009-
49 2541(94)90096-5, 1994.
- 50 Wassenaar, L., Ahmad, M., Aggarwal, P., van Duren, M., Pölsenstein, L., Araguas, L. and Kurtas, T.:
51 Worldwide proficiency test for routine analysis of $\delta^2\text{H}$ and $\delta^{18}\text{O}$ in water by isotope-ratio mass



- 1 spectrometry and laser absorption spectroscopy, *Rapid Commun. Mass Spectrom.*, 26(15), 1641–
2 1648, doi:10.1002/rcm.6270, 2012.
- 3 Wassenaar, L. I., Terzer-Wassmuth, S., Douence, C., Araguas-Araguas, L., Aggarwal, P. K. and Coplen,
4 T. B.: Seeking excellence: An evaluation of 235 international laboratories conducting water isotope
5 analyses by isotope-ratio and laser-absorption spectrometry, *Rapid Commun. Mass Spectrom.*, 32(5),
6 393–406, doi:10.1002/rcm.8052, 2018.
- 7 West, A. G., Patrickson, S. J. and Ehleringer, J. R.: Water extraction times for plant and soil materials
8 used in stable isotope analysis, *Rapid Commun. Mass Spectrom.*, 20(8), 1317–1321,
9 doi:10.1002/rcm.2456, 2006.
- 10 White, w., Arthur and Pichler, E.: Water Sorption Properties of Clay Minerals. (No.
11 208), [https://www.ideals.illinois.edu/bitstream/handle/2142/44988/watersorptioncha266whit.pdf?s](https://www.ideals.illinois.edu/bitstream/handle/2142/44988/watersorptioncha266whit.pdf?sequence=2)
12 [equence=2](https://www.ideals.illinois.edu/bitstream/handle/2142/44988/watersorptioncha266whit.pdf?sequence=2), 1959.
- 13 Zieffler, A. S., Haring, J. R. and Long, J. D.: 12. Unplanned Contrasts, in *Comparing Groups:*
14 *Randomization and Bootstrap Methods Using R*, pp. 255–286, John Wiley & Sons, New York, NY,
15 USA., 2012.
- 16

1 **Tables**

- 2 Table 1: Description of the respective extraction systems that participated in the cryogenic
3 inter-laboratory comparison and the applied extraction parameters for extraction approach I.
4 Note that not every lab provided the same detailed information.

5

Lab no.	Country	Description of CWE facility	Number of extraction slots	Extraction parameters for approach I
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)
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)
3	Germany	Heating lamps; LN ₂ -cold trap	5	Temperature: ~115°C, vacuum: 1 Pa, time: 90 min
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)
5	France	Cold trap: mixture of LN ₂ and EtOH	4	Temperature: 65°C, cold trap: -50--70°C, vacuum: 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: 95-100°C, starting with sealed vacuum of 0.3 Pa, time: 150-180 min
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
8	Germany	Pair of Valco Exetainer® vials connected with a 1.56 mm stainless steel capillary as	12	Temperature: 200°C, vacuum: 50 Pa, time: 15 min



		extraction-collection unit; an aluminum block on a hot plate, LN ₂ -cold trap		
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
10	Switzerland	Glass tubes (Vacutainer), LN ₂ -cold trap, water bath	20	Temperature: 80°C
11	USA	Pyrex Culture Tubes (25mm x 150 mm), volume: 75 ml; heaters: electric coil (only allow to heat 2/3 of the tube)	10	Temperature: 102°C, vacuum: <0.1-2.7 Pa, time: on average 81 min (silty sand), 134 min (clayey loam)
12	Germany	Glass tubes, LN ₂ -cold trap, water bath	8	Temperature: 80°C, vacuum: 600 Pa, time: 60 min
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
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)
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 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	20	Temperature: 80°C, vacuum: 10-350 Pa, time: 30 min (silty sand), 40 min (clayey loam)
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)



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

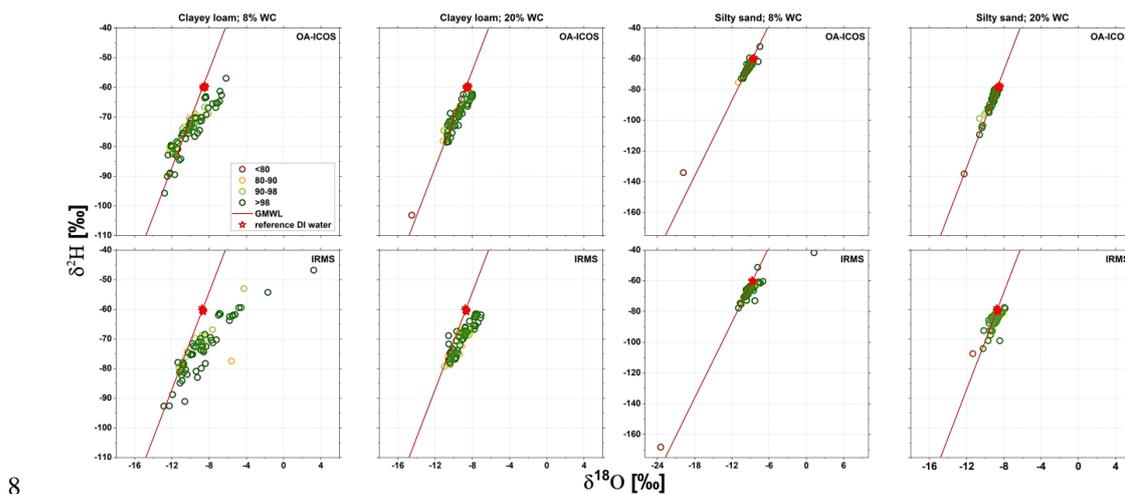
Parameter	Clayey loam	Silty sand
pH-value	7.2 \pm 0.2	5.0 \pm 0.3
Water holding capacity [g 100g ⁻¹]	43.4 \pm 0.8	32.1 \pm 1.4
Organic carbon [%]	2.0 \pm 0.2	0.7 \pm 0.1
Cation exchange capacity [cmol(+) kg ⁻¹]	30.6 \pm 5.1	4.1 \pm 0.6
Particle size [mm] distribution according to German DIN [%]		
<0.002 (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 ₂ O ₃	8.8	3.3
Fe ₂ O ₃	3.1	0.5
MnO	0.1	0.0
MgO	1.5	0.1
CaO	5.3	0.2
Na ₂ O	0.9	0.3
K ₂ O	1.7	1.7
P ₂ O ₅	0.2	0.1
SO ₃	0.1	<0.01
Cl	<0.002	<0.002
F	<0.05	<0.05

8



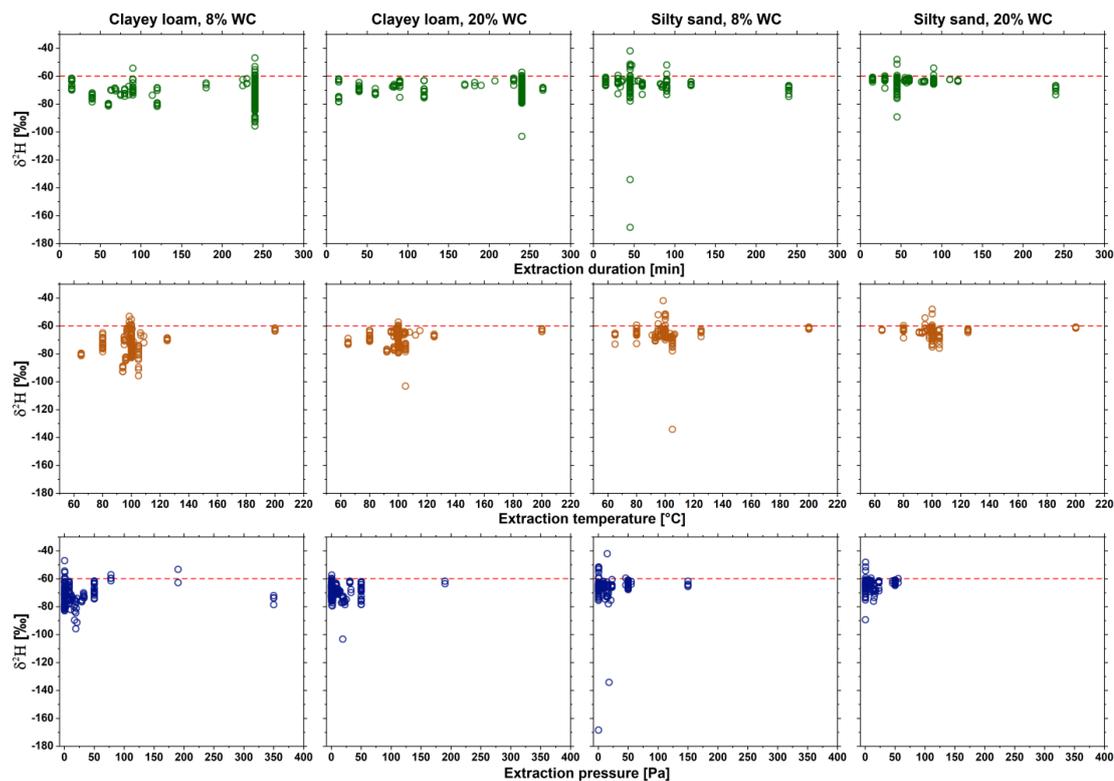
1 Figures

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 results in comparison to the spiked reference DI water (red asterisks)
5 shown in dual isotope space. For reference, plots include the Global Meteoric Water Line
6 (GMWL, solid red line). Water recovery rates are shown for those labs that provided the
7 complete set of soil weight data (in % of previously added water).





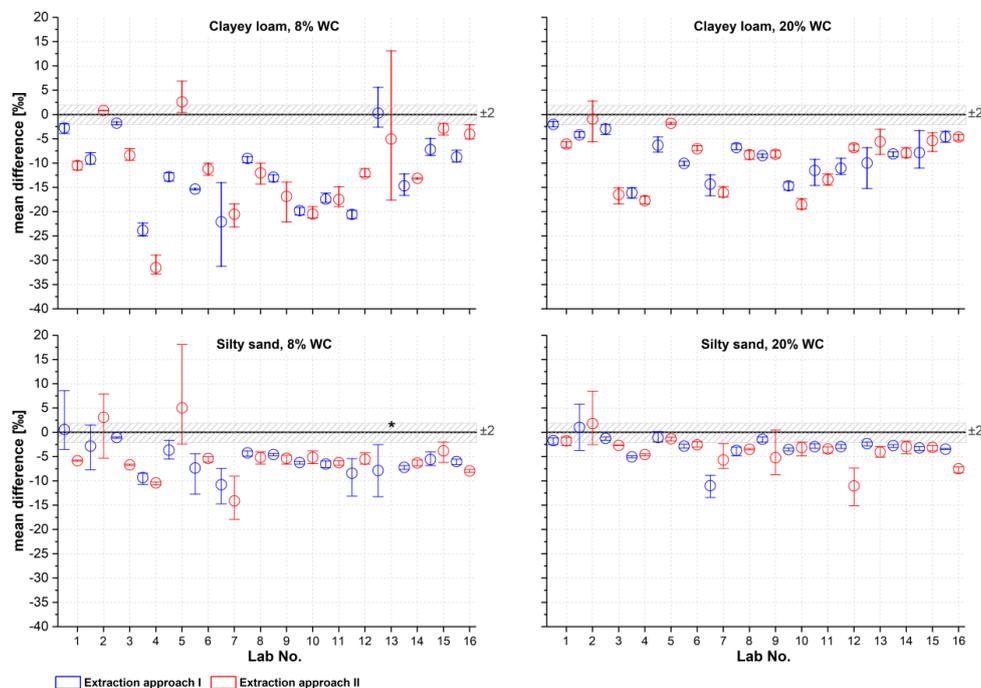
- 1 Figure 2. Effect of cryogenic extraction parameters (duration, temperature, and pressure) on
- 2 $\delta^2\text{H}$ results of both soil types (clayey loam and silty sand) and WCs (8 and 20%) shown for all
- 3 labs. The mean reference DI water $\delta^2\text{H}$ value is included as red dotted line.



- 4
- 5



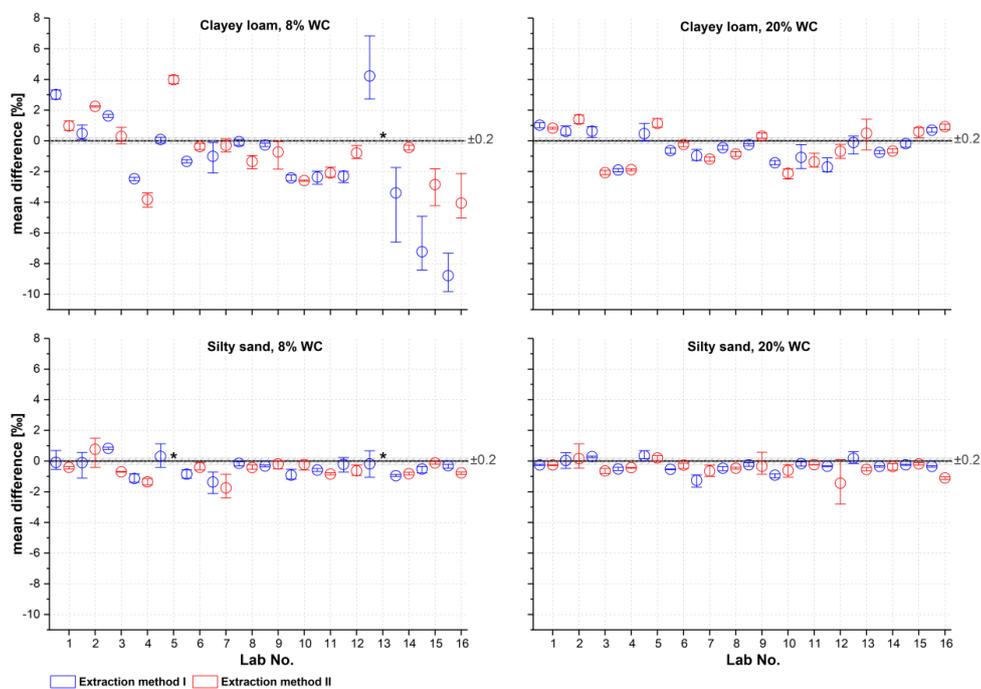
1 Figure 3. Mean differences from reference DI water for $\delta^2\text{H}$ OA-ICOS results of water extracts
 2 from both extraction methods (lab-procedure: I and pre-defined: II), soil types, and water
 3 contents (8 and 20% WC) including TSD of ± 2 for $\delta^2\text{H}$ (Asterisk: -108.4 for $\delta^2\text{H}$). Y-error bars
 4 represent the isotopic variation of the replicates. There were no significant differences between
 5 the two extraction approaches overall labs.



6
 7

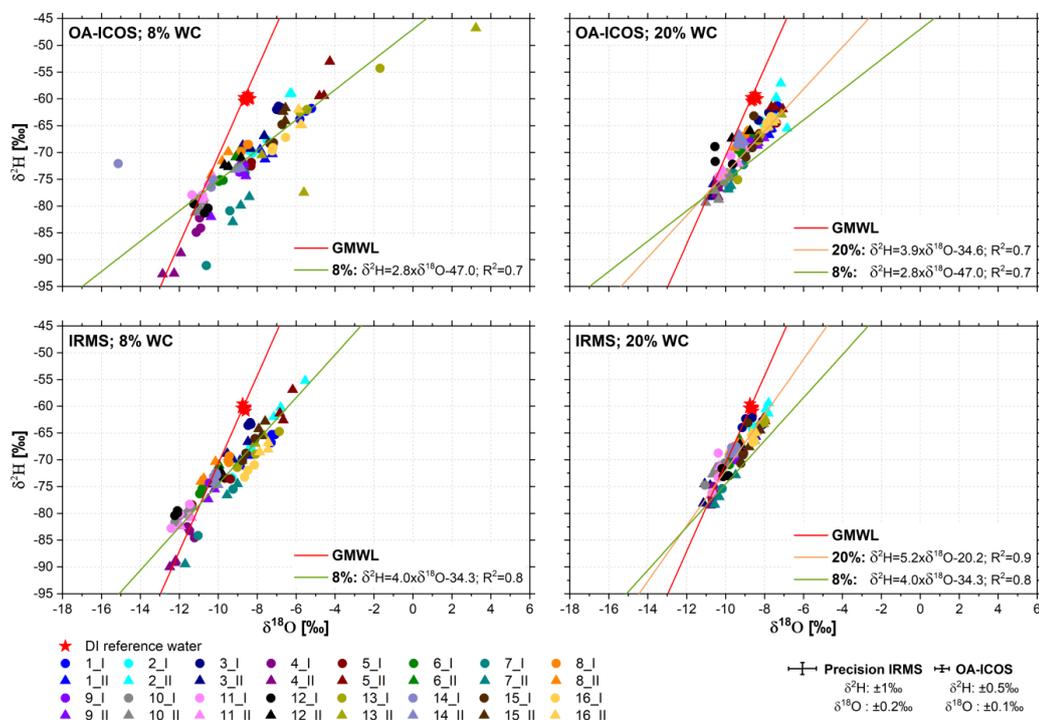


1 Figure 4. Mean differences from reference DI water for $\delta^{18}\text{O}$ OA-ICOS results of water extracts
 2 from both extraction methods (lab-procedure: I and pre-defined: II), soil types, and water
 3 contents (8 and 20% WC) including TSD of ± 0.2 for $\delta^{18}\text{O}$. Asterisks represent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





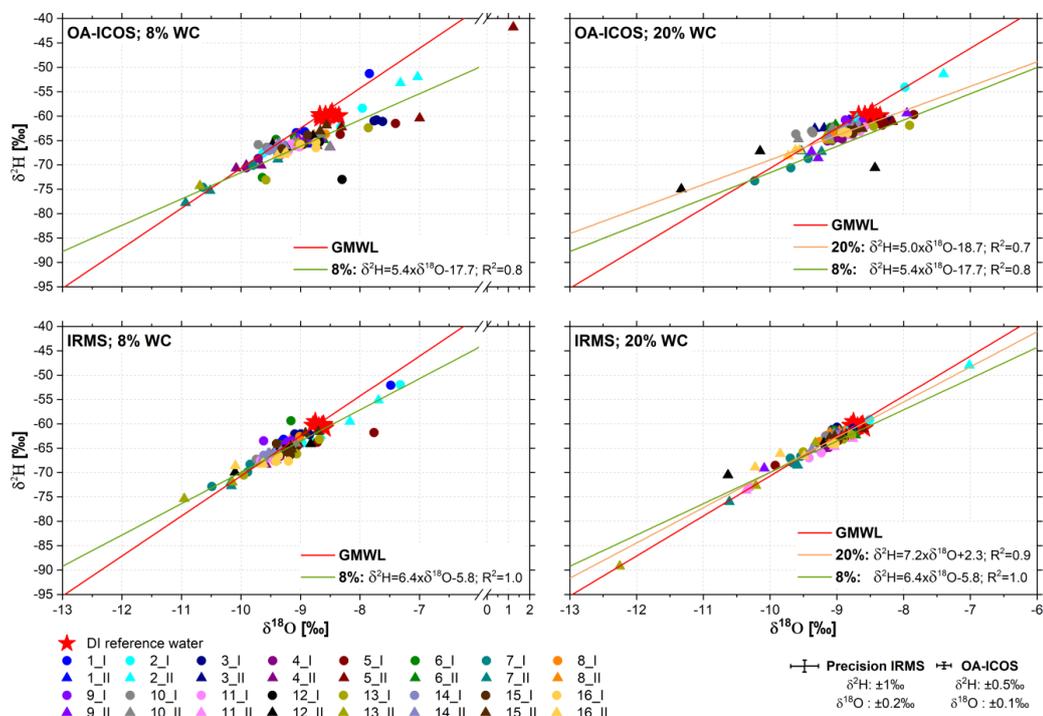
1 Figure 5. Dual isotope plots of clayey loam extracts for 8% and 20% WC in comparison to
 2 reference DI water (red asterisks) for OA-ICOS and IRMS (upper and lower panels,
 3 respectively). For reference, plots include the Global Meteoric Water Line (GMWL, solid red
 4 line) and evaporation water lines for 8% and 20% WC (solid green and orange lines,
 5 respectively).



6
 7



1 Figure 6. Dual isotope plots of silty sand extracts for 8% and 20% WC in comparison to
 2 reference DI water (red asterisks) for OA-ICOS and IRMS (upper and lower panels,
 3 respectively). For reference, plots include the Global Meteoric Water Line (GMWL, solid red
 4 line) and evaporation water lines for 8% and 20% WC (solid green and orange lines,
 5 respectively).

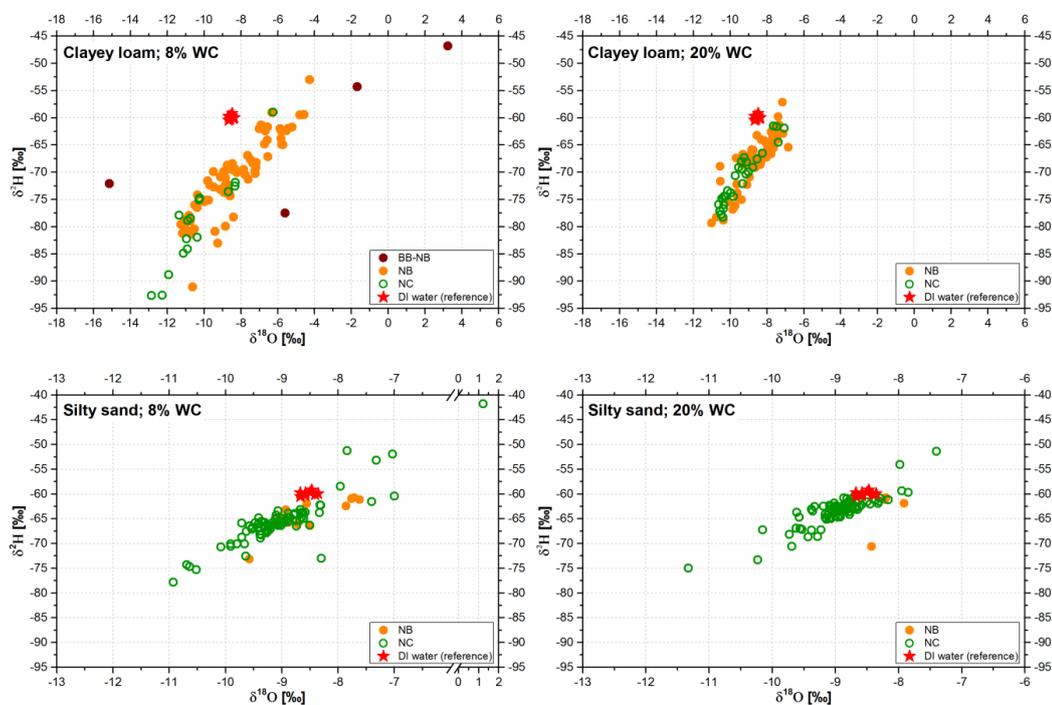


6

7



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



6

7



1 **Appendix**

2 Appendix 1. Cryogenic system – Questionnaire

3

4

Inter-laboratory comparison of CWE systems

5

6

Contact person	<input type="text"/>	<input type="text"/>	
	Last Name	First Name	
Address	<input type="text"/>	<input type="text"/>	
	Street	Street No.	
	<input type="text"/>	<input type="text"/>	<input type="text"/>
	City	Postal Code	Country
8	<input type="text"/>	<input type="text"/>	
	Phone Number	Email	

9

10 **Cryogenic system – Questionnaire**

11

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

13

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

15

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

18

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

20

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

22

<input type="text"/>	<input type="text"/>
Type of plant material (e.g., twig, root crown)	Soil type

23

24

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

26