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Ecohydrology Bearings – Invited Commentary

Critical issues with cryogenic extraction of soil water for stable isotope analysis

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ABSTRACT

Cryogenic water extraction is the most widely used method to facilitate the laboratory removal of soil pore water for isotopic analysis. However, recent studies have suggested that cryogenic extraction conditions (extraction time, temperature, vacuum threshold) and physicochemical soil properties can influence extracted water isotopic signatures. Here, we argue that new work is needed to analyse the full extent of these effects on the extracted water isotopic composition. We illustrate this need with a simple lab experiment and show that in addition to extraction times, soil organic matter and its exchangeable bonded hydrogen fraction influence the resulting isotope composition. We hope these comments stimulate discussion on the assumptions and limitations of cryogenic extraction for soil water and lead ultimately to a standardization of testing approaches. © 2016. The Authors. Ecohydrology published by John Wiley & Sons Ltd.

KEY WORDS soil water extraction; stable water isotope analysis; cryogenic water extraction; cryogenic extraction conditions; soil property isotope effects

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INTRODUCTION

The use of isotope tracers has redefined process hydrology. Much of this has been accomplished with sampling and interpreting liquid water samples of precipitation, streamflow, and groundwater. While soil water has been sampled for decades, it has usually been extracted from field-based suction lysimeters at very low tensions. Consequently, most of our understanding of the age, origin, and flow pathways of water at the catchment scale is through the lens of mobile water. Ecohydrologically focused studies have begun to explore lower mobility water (soil and plant waters) and its link to subsurface mixing and water residence time and its interaction and feedback to ecosystem processes (Asbjornsen *et al*., 2011). Such studies usually rely on laboratory-based cryogenic extraction of water – effectively removing all the water for subsequent isotope analysis.

Recent studies have shown that the isotopic ratios of suction lysimeter waters differ from cryogenically extracted waters (Brooks *et al*., 2010; Figueroa-Johnson *et al*., 2007; Landon *et al*., 1999; Zhao *et al*., 2013). While there is of course a large literature on isotopes in plant ecophysiology (see review articles of Adams and Grierson, 2001; Dawson *et al*., 2002; Newton, 2010), there has been rather little comment on the techniques we use to extract low mobility waters in soils. Physically, we distinguish between water held at suctions less than field capacity (mobile water) and water held at suctions greater than field capacity – usually referred to as plant available water or tightly bound water[1] (Huntington, 2006). Such low mobility water can be found in micropores and thin films around soil aggregates and is relatively stagnant in comparison with mobile water held under less suction (Landon *et al*., 1999). The presence of water in films as well as under concave menisci is most

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[1]Field capacity and permanent wilting point are defined as the volumetric fraction of water in the soil at soil water potentials of 100–333 and 15 000 hPa, respectively. The plant available water is defined as the difference between field capacity and permanent wilting point (Huntington, 2006).

important in clayey soils and at high tensions, and it is influenced by the electric double layer and the exchangeable cations present (Hillel, 2004). This results in a greater volume of low mobility water in clay soils. In contrast, the percentage of low mobility water is less in sandy soils than in more structured soils because adsorption is relatively unimportant and capillarity dominates (Bengtsson *et al.*, 1987; Hillel, 2004). Thus, soils with different textures may behave differently in terms of water mixing processes (Zhao *et al.*, 2013). Many early laboratory, modelling, and field studies examined this physical co-existence of mobile and low mobility waters in the unsaturated zone (Coats *et al.*, 1964; De Smedt and Wierenga, 1984; Gaudet *et al.*, 1977; van Genuchten and Wierenga, 1976). Recent studies have shown that cryogenic extraction conditions (extraction time, temperature, vacuum threshold) and physicochemical soil properties considerably impacted the extracted soil water isotope results (e.g. Meißner *et al.*, 2014; Orlowski *et al.*, 2013). Consequently, there is an urgent need to evaluate cryogenic extraction of soil waters as this technique continues to be the most widely used (Koeniger *et al.*, 2011),[2] and its results are compared with other pools of water in the ecohydrological system. The key question is: how do soil types and their properties affect isotope results obtained under certain cryogenic extraction conditions? In view of the increasing number of laboratories, which now apply cryogenic water extraction, especially in the context of ecohydrological studies, it seems timely to critically examine these issues and develop standardized cryogenic extraction conditions for a variety of soil types. Here, we present a critical overview of cryogenic extraction of soil water and offer a vision for moving forward with this approach.

WHAT IS CRYOGENIC EXTRACTION AND WHAT IS THE PROBLEM?

Cryogenic extraction facilitates the removal of liquid water from soil and plant material for stable isotope analysis of the water. During water extraction, the soil or plant sample is heated (usually at temperatures of 90–100 °C) under vacuum for a prescribed time, causing the water to evaporate from the soil or plant material and then to become trapped via freezing in a (cryogenic) liquid nitrogen cold trap (Ingraham and Shadel, 1992). After defrosting, the liquid water sample is accessible for isotope analysis. Dalton (1989) was among the first to publish a schematic of a cryogenic extraction system. Other early work by Araguás-Araguás *et al.* (1995), Ingraham and Shadel (1992), Jusserand (1980) or Walker *et al.* (1994) first critically explored extractions of different soil types.

Recent studies have exposed issues with cryogenic extraction related to its effect on the isotopic composition of extracted water (Meißner *et al.*, 2014; Orlowski *et al.*, 2013). Nevertheless, the only recent method improvements have been related to reduction of extraction times – enabling a higher sample throughput (Orlowski *et al.*, 2013) – or modifications of the cryo-system's set-up related to downsizing of the overall apparatus, e.g. Koeniger *et al.* (2011). Thus, we have avoided the bigger issue of extraction and soil problems and not achieved any uniformity in the way independent laboratories apply cryogenic vacuum extraction techniques. The biggest problem is that cryogenic extraction of soil water is often not able to recapture a label of know isotopic signature added to the soil prior to extraction. The current general procedure for recovery confirmation is to oven-dry soil samples (105 °C, 24 h) and spike them with a given amount of water with known isotopic composition (similar to Koeniger *et al.* (2011) or Orlowski *et al.* (2013)). The added water should be recoverable, theoretically in terms of gravimetric soil water content and isotopic composition via cryogenic extraction. While simple in theory, water recovery via this approach has been difficult in practice. To our knowledge, only Koeniger *et al.* (2011) and West *et al.* (2006) have shown ability to do this in dual isotope space (^{2}H and ^{18}O), Koeniger *et al.* (2011) for a sandy soil, West *et al.* (2006) for a clayey and sandy soil. Orlowski *et al.* (2013) were only able to recapture the introduced $\delta^{2}H$ isotopic signature from a silty sand and the $\delta^{18}O$ isotopic signature from a highly clayey silt. Goebel and Lascano (2012) showed such capability for $\delta^{18}O$ for sandy clay loam water extracts. Other studies have reported significant differences between the recovered and added reference water (e.g. Araguás-Araguás *et al.*, 1995; Walker *et al.*, 1994).

In general, past and more recent findings suggest that isotopic fractionation effects are more pronounced for soils with a large fraction of small pores (<0.002 mm) (Barnes and Turner, 1998), i.e. clayey soils (e.g. Koeniger *et al.*, 2011; Orlowski *et al.*, 2013). Cryogenic extraction is something of a brute force technique that extracts a mixture of various water pools (Sprenger *et al.*, 2015) having different isotopic composition (e.g. Landon *et al.*, 1999; Sprenger *et al.*, 2015; Zhao *et al.*, 2013). However, the alteration of $\delta^{18}O$ depends strongly on the soil type (Araguás-Araguás *et al.*, 1995). This was underscored by Meißner *et al.* (2014), who observed changes in the $\delta^{18}O$ composition of cryogenically extracted soil water due to clay and/or carbonate content. Oerter *et al.* (2014) also found that cations adsorbed to clay minerals attracted water in the form of hydration spheres. The high cation exchange capacities of clay minerals create isotopically distinct water

[2]Although many new soil and/or plant water extraction techniques have been developed, e.g. the water vapour equilibrium method (Hsieh *et al.*, 1998; Wassenaar *et al.*, 2008), Picarro's Induction Module (Picarro, 2015), the micro-wave technique (Munksgaard *et al.*, 2014), *in situ* monitoring of soil pore water (Rothfuss *et al.*, 2013; Volkmann and Weiler, 2014), or the accelerated solvent extraction technique (Zhu *et al.*, 2014).

pools that may not be completely mixed with other water pools of the bulk water in the soil solution[3] (Oerter *et al*., 2014). This may cause a distinct drift of isotopic signatures of the extracted soil water. Araguás-Araguás *et al*. (1995) and Ingraham and Shadel (1992) showed that at low water contents, the fraction of interlayered water becomes more pronounced during soil water extraction. The isotopic fractionation effect increases because of the formation of hydration spheres around cations, which leads to a fractionation of oxygen isotopes of water[3] (Sofer and Gat, 1972). Meißner *et al*. (2014) also found that the presence of carbonates significantly altered the $\delta^{18}O$ isotopic composition of added water, whereas the shift in $\delta^{2}H$ values between added and extracted water was independent from the carbonate content.

In addition, extraction time is another influencing factor. In general, the fraction of light isotopes is known to be extracted first, and fractionation effects appear to be more pronounced for lower mobility water, which is obtained towards the end of the extraction process (Barnes and Turner, 1998). West *et al*. (2006) recommended extraction times to obtain an unfractionated water sample for sandy and clay soils of 30 and 40 min, respectively, similar to Goebel and Lascano (2012) who suggested 30 min extraction duration for a sandy clayey loam. Jia *et al*. (2012) applied minimum extraction times of 40 to 45 min for loamy soils and 35 min for sandy soils. Koeniger *et al*. (2011) utilized a modified apparatus set-up with even shorter extraction times (2·5 to 40 min), recovering the original water isotopic signature after 15 min from a sandy soil. Orlowski *et al*. (2013) applied extraction durations of up to 180 min. Mora and Jahren (2003) extracted soil samples for 360 min. However, the extraction duration strongly depends on the soil type and water content, and is very different for each extraction system. For dry soils, Walker *et al*. (1994) found that the extraction could lead to large errors in the isotopic composition. Thus, Geris *et al*. (2015) adjusted extraction times to >120 min because of higher water contents in their soils (Histosols and Podzols) using a cryogenic system set-up similar to West *et al*. (2006). Nevertheless, if the distillation process is not conducted until completion, considerable Rayleigh fractionation can occur (Goebel and Lascano, 2012) – meaning the relation between the isotopic composition of a liquid water reservoir and the evaporating vapour (Dansgaard, 1964). These isotopic fractionation results in a bias towards more positive δ-values in the extracted water sample (Barnes and Turner, 1998). More recently, Orlowski *et al*. (2013) showed that even if the extraction is conducted until completeness (in terms of weight), the original added known isotopic signature may not be recovered from different soil types.

Beyond extraction duration, extraction temperature can impact the success of recovering the added isotopic composition. Araguás-Araguás *et al*. (1995) showed early that a reservoir of weakly bound soil water exists especially in clayey soils (interlayered water), which remains largely intact at extraction temperatures <100 °C and is isotopically different from the mobile water (Araguás-Araguás *et al*., 1995). To keep the effect of interlayered water as low as possible and if the isotopic composition of mobile soil water is of interest, they recommended lower temperatures and advocated for shorter extraction times. So far, no attempt has been made to separate the different soil water reservoirs via cryogenic vacuum extraction depending of the type of soil water of interest (mobile to lower mobility water). Still, only few studies applied extraction temperatures >100 °C (Walker *et al*., 1994; Araguás-Araguás *et al*., 1995; Palacio *et al*., 2014). Despite using such high temperatures, Walker *et al*. (1994) could not recover the reference water added to dry and wet clays, sand, and gypseous sand. They concluded that decomposition of organic matter or extraction of crystallization water could have affected the isotope results. However, these early high-temperature extractions yielded smaller deviations from the isotopic signatures of the introduced water compared with low-temperature extractions (35 to 80 °C) (Walker *et al*., 1994). Araguás-Araguás *et al*. (1995) achieved recovery rates >98% for pure sand by either increasing the temperature or the extraction time. More recently, Palacio *et al*. (2014) tested the ability of high-temperature extractions (120 °C) on recovering various types of labelled water from gypsum soils. Nevertheless, the isotopic signature of all differently labelled water additions (boiled, snow, and D_2O-labelled water) varied from the extracted isotopic signature and the calculated mother solution from which the gypsum originally crystallized. Especially, water extracted at intermediate temperatures (50 °C) showed inconsistent values as compared with the labelled water additions (Palacio *et al*., 2014). In general, the applied temperatures during the extraction are likely to mobilize both hygroscopic (Koeniger *et al*., 2011) and biologically bound water (Sprenger *et al*., 2015).

In addition to temperature, extraction pressure can have an effect. Extraction pressures ranging from 13 Pa (Goebel and Lascano, 2012), 8·0 Pa (West *et al*., 2006), 3·07 Pa (Koeniger *et al*., 2011), 1·3 Pa (Vendramini and Sternberg, 2007), 1·0 Pa (Palacio *et al*., 2014) to 0·13 Pa (Peters and Yakir, 2008) have been applied. Even lower vacuum levels (<0·1 Pa) have been suggested for obtaining the isotopic signature of the added water (Orlowski *et al*., 2013).

[3]Exchangeable cations on the clay surfaces hydrate and reconfigure into inner-sphere and outer-sphere complexes of the electrical double layer; some portion of the cations goes into solution by becoming fully solvated ('salinity isotope effect'). Those cations that are not fully solvated remain in the inner-sphere and outer-sphere complexes adsorbed to the clay surfaces and form hydration spheres around them. This leads to a different isotope fractionation effect on the bulk soil water (Oerter *et al*., 2014).

However, effects of different vacuum thresholds over a broad range of different sample media on the cryogenically extracted soil water isotopic signatures have not been thoroughly tested.

Lastly, soil type and structure can influence extracted water isotopic signatures. Again, early work commented on this (Ingraham and Shadel, 1992; Walker *et al*., 1994; Araguás-Araguás *et al*., 1995). Araguás-Araguás *et al*. (1995) reported systematic deviations of the extracted water from the added labelled water. The water extracted from a soil with very high clay content was depleted by approximately 5·2‰ for δ^2H and 0·36‰ for δ^{18}O in comparison with the reference water. For soils with medium clay content, this average depletion was 9·6‰ for δ^2H and 0·47‰ for δ^{18}O, respectively. Koeniger *et al*. (2011) worked with multiple soils and tree cores and showed deviations of up to 3·3‰ for δ^2H and 0·42‰ for δ^{18}O of spiked samples. Their method seemed to work well for sandy soils, but there appeared to be some residual water in the spiking experiment with tree cores, silt, and clay-rich soils. Moreover, Orlowski *et al*. (2013) showed strongly depleted isotopic signatures of extracted water from a clayey loam, which were statistically significantly different from the added water ($p < 0{\cdot}05$).

A SMALL DEMONSTRATION EXPERIMENT

To demonstrate these effects on recovery, we present a simple experiment. Six different soil types (three replicates per soil material) representing a gradient from sandy to loamy soils were chosen as testing materials. Among them were two silty sands, a loamy sand, and a clayey loam from the German State Research Institute for Agriculture (LUFA Speyer: German State Research Institute for Agriculture, Speyer, DE, 2015); a local fine sand from Homberg-Ohm (Hesse, Germany), and an Ah-horizon soil from a Luvisol (highly clayey silt) collected at the Schwingbach catchment (Hesse, Germany). Disturbed soil samples were sieved (2 mm), homogenized, oven-dried (105 °C, 24 h), and rehydrated with local tap water (δ^2H: $-59{\cdot}4 \pm 0{\cdot}8$‰, δ^{18}O: $-8{\cdot}6 \pm 0{\cdot}2$‰; $N = 28$) to a gravimetric water content of 20%. To ensure homogeneity, rehydrated soil samples were equilibrated for 5 days in hermetically sealed tubes in a desiccator at room temperature (19 °C). Soils were cryogenically extracted for 120 and 180 min at 95 °C applying a static vacuum of 0·1 Pa (following Orlowski *et al*., 2013). Gravimetric soil water analyses before and after water extraction as well as after oven-drying of the extracted soils (105 °C, 24 h) revealed complete water extraction in terms of weight. Isotopic signatures were analysed at the Institute for Landscape Ecology and Resources Management (Justus Liebig University Giessen) according to the International Atomic Energy Agency (IAEA) standard procedure (Newman *et al*. 2009) utilizing a Los Gatos Research DLT-100-Liquid Water Isotope Analyzer (Los Gatos Research Inc., Mountain View, CA, USA). Isotopic ratios are reported in per mil (‰) relative to the Vienna Standard Mean Ocean Water (Craig, 1961). Precision of analyses was ±0·6‰ for δ^2H and ±0·2‰ for δ^{18}O (LGR: Los Gatos Research, 2013).

Figure 1 (inset) shows how the cryogenically extracted δ^2H values are affected by soil physicochemical properties. The δ^2H values significantly correlate and become progressively lighter with increasing organic carbon content. The same was true for water holding capacity (field capacity) and nitrogen content (data not shown). Although applied extraction conditions are consistent with common literature values (see previous paragraph on extraction condition effects), soils containing a considerable proportion of small pores (e.g. clayey loam: 26·3 ± 2·1% pore size <0·002 mm) show great deviations from the isotopic values of the added reference water (dashed red line). In contrast, sandy soil water extracts have a similar isotopic composition as the added reference water. For the 120 min extraction time, the loamy sand samples deviate from the reference water on average by 8·9‰, the clayey loam samples by 11·7‰ for δ^2H. At longer extraction times (180 min), slopes and intercepts of the linear regression lines (Figure 1, inset) tend to be smaller, which results in less depleted isotopic signatures of the 180 min extracts. No statistically significant correlations between cation exchange capacity or pH values and recovered isotopic signatures were found. However, naturally occurring co-correlations between cation exchange capacity and clay content as well as between water holding capacity and organic carbon content were observed. Figure 1 also shows the extraction results in dual isotope space. Again, the same gradient of isotopic deviation from the reference water could be observed with the clayey loam plotting furthest from the reference water (red dot) and the sandy soil showing the smallest deviation from the spiking water.[4]

A VISION FOR MOVING FORWARD

What to do immediately?

The first step is to recognize that there is a problem with the technique! We have known of these 'issues' with cryogenic extraction for 20 years. Nevertheless, it is still the standard method for extraction of soil and plant water in ecohydrology. We hope that this critical evaluation of the technique shows that continued non-standardized use of cryogenic extraction is problematic. Soil properties and/or extraction conditions should be assessed and reported in

[4]Note that the dual isotope plot combines multiple effects on the extracted soil water isotopic composition.

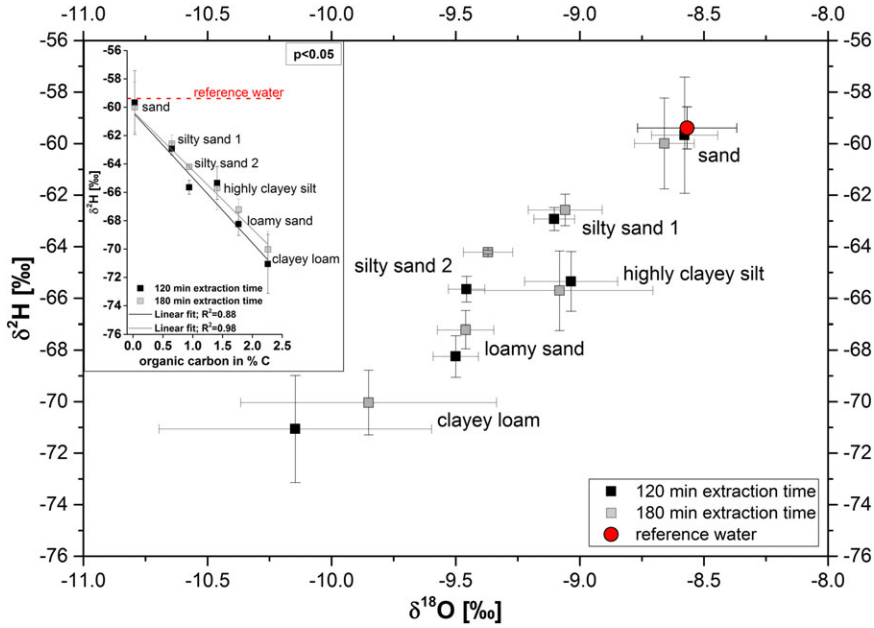

Figure 1. Dual isotope plot of the soil water extraction results for six different soil types extracted at 120 and 180 min in comparison with the added reference water (red symbol). X-error and Y-error bars represent the isotopic variation of the three replicates per soil type. The inset in the upper left-hand corner shows the effect of organic carbon content on cryogenically extracted δ^2H results for the same soil types and extraction times. Y-error bars represent the isotopic variation of the three replicates, respectively.

future studies. With the past studies, it remains difficult for *ex post facto* correction in terms of soil property or extraction conditions effects because such information is rarely reported, and large variability in the details of the approach are common (Walker *et al*., 1994). We encourage the isotope community to determine soil properties prior to cryogenic water extraction to ensure transparency of obtained results and to make potential soil property effects known. Because cryogenic extraction systems differ in their set-up (e.g. size of extraction container, dynamic or static vacuum application, type of heating element), we further suggest that validation of the functionality and reliability of the cryogenic vacuum system be made via tests similar to those reported by Koeniger *et al*. (2011) or Orlowski *et al*. (2013). Additionally, test extractions should be performed to calibrate the extraction parameters to the specific sample material of interest before applying any given set of extraction conditions. Such extraction conditions and soil specifications should be reported in all work because the extracted water isotope results appear to be a function of these extraction process conditions – the extraction system itself along with soil type and water content.

What experiments are needed?

Research is needed to further analyse the influence of soil organic matter, i.e. exchangeable bonded hydrogen (Meißner *et al*., 2014) in organic-rich soils on the cryogenically extracted isotopic composition. Different exchangeable (labile) hydrogen fractions exist in environmental organic matter (O-bonded, N-bonded, and S-bonded or aromatic hydrogen), which interact with ambient water or water vapour (Ruppenthal *et al*., 2010). The labile hydrogen fraction equilibrates isotopically with atmospheric water vapour within minutes (Filot *et al*., 2006; Wassenaar and Hobson, 2000) at temperatures above 100 °C and within days at a temperature of 0 °C (Feng *et al*., 1993). Depending on the concentrations of soil organic matter, there are varying fractions of exchangeable organic and inorganic hydrogen in bulk soil samples (Ruppenthal *et al*., 2010), which must be considered isotopically, e.g. 30% of the hydrogen atoms in cellulose are exchangeable bonded to oxygen (Filot *et al*., 2006). We do not know if the labile hydrogen fraction causes isotope fractionation during cryogenic extraction, i.e. labile hydrogen being released after varyingly long extraction times.

Further, the effects of soil microbiological activities on the extracted water isotope results are poorly known. It is generally assumed that the extent of the bacterial effect on the bulk soil water isotopic composition is more pronounced for soils with small pores, because small-sized fractions contain the most microbial biomass (Jocteur Monrozier *et al*., 1991; Kanazawa and Filip, 1986; Kögel-Knabner *et al*., 2008). For instance, clay-sized particles have a higher surface area than coarser particles, which enables bacterial growth as well as adherence and protection of microorganisms and extracellular enzymes

(Kögel-Knabner *et al*., 2008). With regard to the cryogenic water extraction, temperature-resistant soil bacteria can indeed endure the extraction process (Koeniger *et al*., 2011). The evolved elevated CO_2 concentration during bacterial growth can further cause errors when measuring isotopic values via isotope-ratio mass spectrometry (the respired CO_2 will superpose the added CO_2/He mixture during isotope analysis) (Koeniger *et al*., 2011). This raises the question of whether soil samples should be sterilized prior to water extraction or isotope analysis. Experiments are sorely needed in this regard.

The need for an inter-laboratory comparison

Early studies revealed laboratory-dependent and more recently soil property-dependent isotope results obtained through cryogenic water extraction (Araguás-Araguás *et al*., 1995; Ingraham and Shadel, 1992; Meißner *et al*., 2014; Orlowski *et al*., 2013; Walker *et al*., 1994). As yet, no effort has been made to launch an inter-comparison between different laboratories applying cryogenic vacuum extraction. For the analysis of liquid stable water isotope samples, a common laboratory protocol exists from the IAEA (Newman *et al*. 2009). In contrast, we are not aware of any standard cryogenic extraction protocol. There is an urgent need for a similar standard operating procedure across cryogenic water extraction systems. This standard protocol should specify extraction conditions (duration, temperature, pressure) for different soil types with various properties.

As a first step to generate comparable and consistent isotope results from different cryogenic extraction systems, a worldwide inter-laboratory round robin test could compare the performance of the cryogenic extraction systems and improve the quality standards of the method itself. Additionally, the capability of cryogenic extraction systems to recover water from a set of different soil types and water contents could be examined. The design of such a worldwide inter-laboratory trial could be similar to the inter-laboratory comparison for δ^2H and δ^{18}O analysis of liquid water samples (Water Interlaboratory Comparison exercise, WICO) (Wassenaar *et al*., 2012), which is regularly performed by the IAEA. Instead of testing routine measurements of δ^2H and δ^{18}O in water via isotope-ratio mass spectrometry and laser absorption spectroscopy, isotope results gained through different cryogenic soil water extraction systems will be inter-compared. Another option could be to define two physicochemically different standard soil types, labelled with water of a pre-defined stable water isotope composition. These could be extracted cryogenically under defined conditions as a benchmark test for every cryogenic extraction system. All future cryogenic extraction applications could then report on this benchmark test, allowing groups who intend to set up such an extraction facility to test their system against known standards.

SUMMARY

We have shown – with our small literature review and our experiment – the link between cryogenically extracted soil water isotopic signatures and different soil physicochemical properties. This is a problem because natural field soils are amalgamations of different soil textures and represent a distribution of pore sizes. Soil property 'effects' as shown in Figure 1 have broad implications for interpretations of extraction results past and future. Therefore, estimates of plant water uptake depths, use of a soil water end member in hydrograph separation, or mean residence time analysis should be critically examined in light of the different mobilities of water in different soil types and pore spaces. Research is urgently needed to determine whether soils that showed large deviations from the reference water in our small trial, i.e. clayey and/or loamy soils, are even suitable for cryogenic vacuum extraction given these effects or if cryogenic extraction parameters (time, temperature, vacuum threshold) can be adapted to improve the isotopic recovery of these soils. Such soil waters may be better extracted or analysed via the water vapour equilibrium method, but, of course, inter-comparison of the different extraction techniques is first needed.

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