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## Critical issues with cryogenic water extraction for tracing plant's source water

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Numerous scientists and disciplines around the world are applying stable water isotope techniques—, especially in the ecohydrological context. For more than two decades, cryogenic vacuum extraction has been the most widely used method for obtaining water from soils and plant tissues for isotope analysis. Recent findings suggested that cryogenic extraction conditions (extraction time, temperature, vacuum threshold) and physicochemical soil properties considerably affected the extracted soil water isotope results. The key question therefore is: Which soil water pool/s are we actually extracting cryogenically under certain extraction conditions and is this soil water pool the source of plant water uptake?

We conducted a greenhouse trial with two different plant species grown on two physicochemically different soils (sandy soil and clayey loam) to test the effects of varying cryogenic extraction conditions and physicochemical soil properties on extracted soil water isotope results. We further aimed to identify the unique soil water isotopic signature which mirrors plant's water source. We sampled root crowns and an aliquot of the first and second soil layer for cryogenic water extraction. To determine the plant water available soil water pool/s, we varied water extraction parameters (time and temperature).

Our dual-isotope study showed that physicochemical soil properties (i.e. clay content, pore size) along with extraction parameters lead to isotope fractionation effects of soil water. Extraction temperature and time significantly impacted isotope results of clayey loam samples but no effect could be observed for the sandy soil. In general, for water extracts of both soil types, longer extraction times and higher temperatures resulted in enriched isotopic signatures, although this influence was more pronounced for the clayey loam. Determining ideal soil water extraction parameters to identify plant available soil water pools revealed that extraction settings of 200°C and <180 min worked best for the clayey loam. Various cryogenic extraction parameter settings were suitable to recover plant's source water from the sandy soil independently from the soil layer but dependent on which isotope (2H or 18O) was considered.

Our study indicated that extracted water isotope results appear to be a function of extraction conditions along with soil physicochemical properties. These findings have broad implications for interpretations of extraction results, especially in the light of plant water uptake depths calculations, use of a soil water component for hydrograph separation, or mean transit time estimation. 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 develop a standard extraction protocol for extraction settings in order to generate meaningful and comparable isotope results.