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Water extraction times for plant and soil materials used in stable isotope analysis

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Stable isotopic analysis of water for many ecological applications commonly requires extractions of water from dozens to hundreds of plant and soil samples. With recent advances in mass spectrometry, water extraction, rather than the isotopic analysis itself, is the bottleneck in sample processing. Using cryogenic vacuum distillation, we have created extraction timing curves to determine how much time (T_{min}) is required to extract an unfractionated water sample. Our results indicated that T_{min} values are 60 to 75 min for stems, 40 min for clay soils, 30 min for sandy soils and 20 to 30 min for leaves. While the extraction times reported here may allow for some reductions relative to times reported in the literature, the extraction process will continue to be a rate-limiting step in plant water analyses. Ultimately, technological advances eliminating the need for extraction are required to greatly increase throughput rates in water isotope analysis for ecological research. Copyright © 2006 John Wiley & Sons, Ltd.

Studies analyzing the stable isotopes of water extracted from plants and soils have become routine in the ecological literature.[1,2] Applications have included, but are not limited to, determining water sources of plants,[3] exploring plant responses to precipitation changes,[4] partitioning evapotranspiration,[5] and examining groundwater recharge.[6] In any given study, dozens to hundreds of water samples are acquired and analyzed because of the temporal and spatial variations that are typically observed in natural ecological systems. Over the past four decades a variety of extraction techniques have been used,[7] including mechanical pressing,[8,9] displacement through centrifugation with an immiscible liquid,[10] microdistillation with zinc,[11] azeotropic distillation with kerosene or toluene,[12] and cryogenic vacuum distillation.[9,13–15] Of these methods, azeotropic distillation and vacuum distillation have been the most commonly employed. Previous studies have shown that the two methods are comparable and accurate, with the possible exception of extractions from dry soils that have a high proportion of bound water.[7,16,17] As a result, vacuum distillation has been increasingly gaining favor for its relative simplicity and the lack of necessity for addition of a solvent.

During vacuum distillation, water is evaporated from the sample and condensed in a collection tube. The isotopic signature of water condensing in the collection tube follows a Rayleigh distillation curve[16] and, therefore, theoretically, the water extraction must proceed to completion to obtain an unfractionated water sample. Such extractions can be time-consuming, requiring on the order of 1 to 16 h to reach completion.[9,16–18] In practice, however, a complete extraction may not be necessary. It has been shown that collecting 98% of the water results in an unfractionated sample for sandy soils.[17] There has been no comparable study that we are aware of for other materials, nor has there has been a clear indication of minimum extraction times required to obtain an unfractionated sample from a material of interest.

Ecological isotopic studies have expanded in spatial and temporal scope as have their demands on analytical capacity. Technical advances in mass spectrometry have allowed rapid, accurate analyses of δ^2H and $\delta^{18}O$ in water.[19] As a result, for studies involving isotopic analysis of water from porous materials, the bottleneck in analysis time is the extraction of water from the material of interest, rather than the isotopic analysis of water itself. Improving the rate of water extraction would reduce the bottleneck in sample processing. In this study, we determined the minimum extraction times required to obtain an unfractionated water sample from plant and soil materials. We discuss our results together with other potential methods for improving the efficiency of the stable isotope analysis of water.

EXPERIMENTAL

Extraction methodology and technical description

Water was extracted from all materials by cryogenic vacuum distillation. The cryogenic distillation apparatus consisted of six independent glass units all attached to a 1-inch stainless-steel vacuum manifold (Fig. 1). Each unit consisted of 3/8-inch glass arm connected to the manifold via a Varian® 801 vacuum gauge and could be isolated from the manifold

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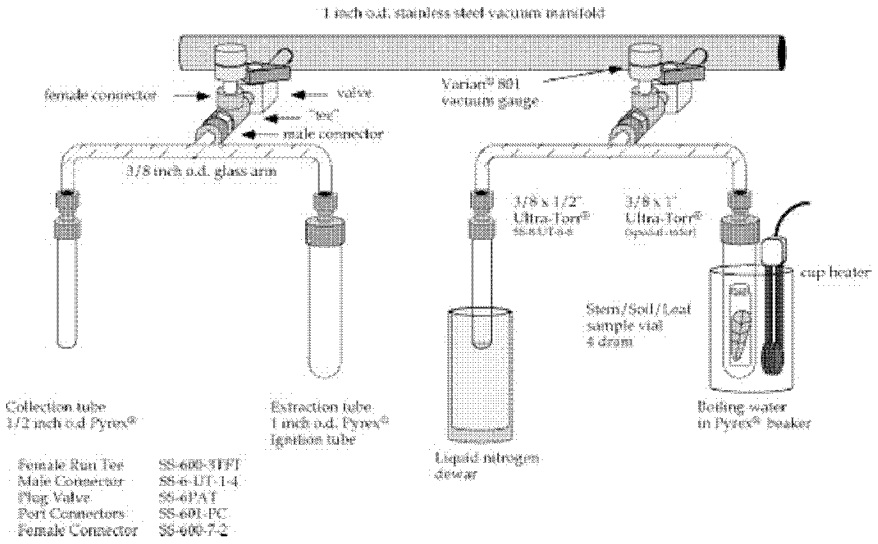

Figure 1. Schematic of the cryogenic extraction line used in this study. See text for detailed description.

by a NUPRO® plug valve. Attached to either end of the 3/8-inch glass arm was a collection tube (1/2-inch Pyrex®) and an extraction tube (1-inch Pyrex®). All connections were made with appropriately sized Ultra-Torr® vacuum fittings or Swagelok® pipe connectors (see Fig. 1 for part numbers). The entire vacuum line was connected to a vacuum pump (Edwards #5).

To extract water from a sample, a previously evacuated unit was isolated from the vacuum manifold. The extraction tube was removed and a sample vial, containing the material of interest, was placed directly inside. The extraction tube was then reconnected to the unit and immersed in liquid nitrogen, freezing the sample and any water vapor in the unit. For samples containing soils, glass wool was packed above the sample vial to prevent the spread of soil particles through the unit. Care was required to ensure that filaments of glass wool did not interfere with the O-ring and compromise the vacuum seal of the extraction tube. Once the sample was frozen, the entire unit was pumped down to a pressure of approximately 60 mTorr. At this point the valve isolating the unit from the vacuum manifold was closed and the pressure in the isolated unit was monitored. If the vacuum was maintained, the Dewar containing liquid nitrogen was removed from the extraction tube and was replaced with a beaker filled with water and containing a heating element. This water was maintained at boiling point throughout the duration of the extraction. Periodic additions of water were required to keep the water level constant. The Dewar of liquid nitrogen was placed on the collection tube in order to freeze out the water vapor emanating from the sample. At the completion of the distillation, the boiling water and liquid nitrogen were removed from the collection tube and the extraction tube, respectively. The collection tube was removed, sealed with Parafilm® and allowed to thaw. The water was then pipetted into a storage vial and was retained for isotopic analysis. The water extracted from coniferous stems and leaves sometimes contained organic compounds that gave the water a milky appearance and a strong odor. In these cases, activated charcoal was added to the extract to adsorb these compounds. The water was then filtered prior to isotopic analysis.

For the purposes of our study, extractions were timed from the moment the extraction tube was placed in the boiling water until the collection tube was removed from the line.

Isotopic analysis

Isotopic analysis was performed at the SIRFER facility (University of Utah, Salt Lake City). Microliter quantities of water were injected directly into a temperature conversion/elemental analyzer (TC/EA) coupled to a Delta Plus XL isotope ratio mass spectrometer (ThermoFinnigan).[19] Hydrogen and oxygen isotope ratios are both obtained from the analysis. Isotope ratios are expressed in ‰ as:

$$\delta^{N}E = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000 \quad (1)$$

where N is the heavy isotope of element E and R is the ratio of the heavy to light isotope ($^{2}H/H$ or $^{18}O/^{16}O$). The δ values are reported relative to V-SMOW. Long-term precision is 1.56‰ ($\delta^{2}H$) and 0.19‰ ($\delta^{18}O$).

Extraction timing curves

We sought to create extraction timing curves for several representative materials of ecological interest (soil, leaves and woody stems). We define an extraction timing curve as the plot of extraction time versus isotopic ratio of extracted water (see Figs. 2–4). When the slope of this curve reached zero, a constant isotopic value had been achieved. We interpret this point (T_{min}) as the minimum time required for an extraction. T_{min} was assessed visually from the extraction timing curves. In order to generate these curves, multiple isotopically uniform samples of a single material were required (see below). These samples were then vacuum-distilled for different periods of time, the extracted water analyzed for $\delta^{2}H$ and $\delta^{18}O$, and the extraction timing curve plotted.

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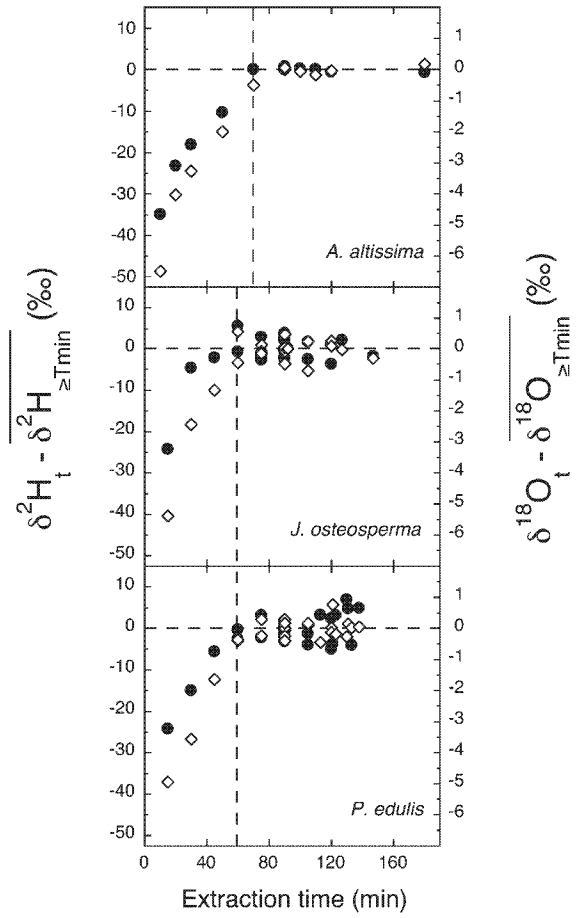

Figure 2. Extraction timing curve for water extracted from suberized stems of three species of woody plants. ● $\delta^{18}O$, ◇ δ^2H. Horizontal dashed line indicates equilibrium value. Vertical dashed line indicates minimum extraction time required for an unfractionated water sample (T_{min}).

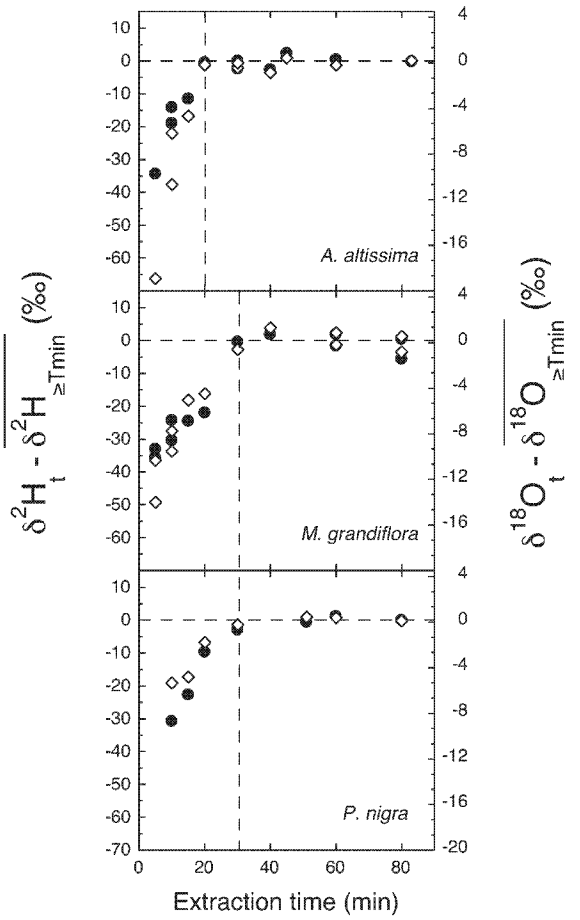

Figure 3. Extraction timing curve for water extracted from leaves of three species of woody plants. ● $\delta^{18}O$, ◇ δ^2H. Horizontal dashed line indicates equilibrium value. Vertical dashed line indicates minimum extraction time required for an unfractionated water sample (T_{min}).

We attempted to obtain isotopically uniform samples for each material as follows.

Soils

Soils were oven-dried (70°C) and then doped with water of a known isotopic composition to close to field capacity in the laboratory. The soils were homogenized, to ensure an even moisture content, and several subsamples were collected in vials and sealed with Parafilm® until analysis.

Leaves

Leaf samples were collected from single, well-lit branches of *Ailanthus altissima* (Mill.) Swingle, *Magnolia grandiflora* L. and *Pinus nigra* Arnold. For the compound-leafed *A. altissima*, leaflets were detached and randomly divided into separate vials, with two leaflets per vial, and sealed with Parafilm® until analysis. For the large-leafed *M. grandiflora*, several leaves were brought back to the laboratory in a plastic bag. The leaves were sliced into small pieces and mixed. From this pool of leaf fragments, individual vials were filled and sealed with Parafilm® until analysis. For the *P. nigra* needles, several fascicles were sampled from the same branch. Needles were detached and randomly divided into separate vials, with two needles per vial, and sealed with Parafilm® until analysis.

Stems

Stem samples were collected from three tree species (*A. altissima*, *Juniperus osteosperma* (Torr.) Little and *Pinus edulis* Englmn.). Stems were excised from fully suberized lateral shoots[18] subtending well-lit, healthy foliage. All stems were sampled upstream of any foliage and were of sufficient length to yield six segments of ~3 cm each. Tests indicated no isotopic difference between segments from a stem, justifying our assumption of isotopic uniformity (data not shown). Each ~3 cm stem segment was cut into ~0.5 cm lengths, collected in a vial and sealed with Parafilm® until analysis.

A minimum of ten extraction times was required to complete an extraction timing curve. This often required more material than could be sampled from an isotopically uniform source (e.g. more than one continuous stem). In such cases, data for the final extraction timing curve were collected in subsets. Each subset came from an isotopically uniform source and contained at least one extraction time that was greater than the minimum extraction time required for an unfractionated sample (T_{min}) thereby allowing us to combine the subsets. Data from the various subsets were

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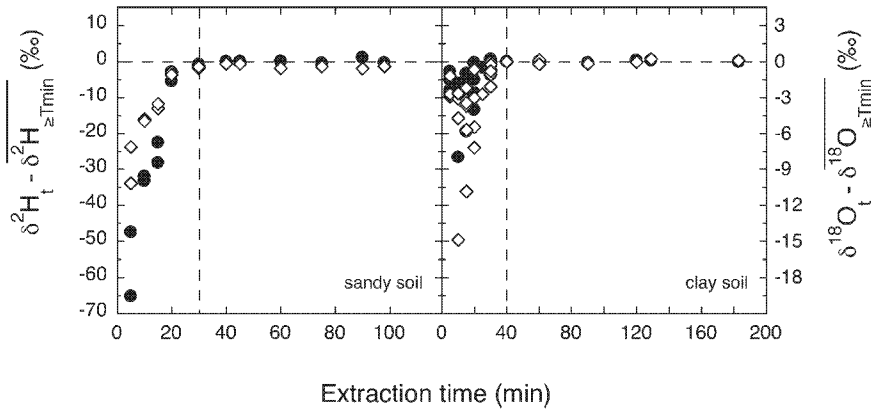

Figure 4. Extraction timing curve for water extracted from a sandy soil and a clay soil. ● $\delta^{18}O$, ◇ $\delta^{2}H$. Horizontal dashed line indicates equilibrium value. Vertical dashed line indicates minimum extraction time required for an unfractionated water sample (T_{min}).

rendered comparable by normalizing to the mean of all samples extracted for a time longer than T_{min}:

$$\delta_{NT} = \delta_T - \overline{\delta_{>T_{min}}} \quad (2)$$

where δ_{NT} = normalized isotope value for a sample extracted for time T, δ_T = isotope value obtained for extraction time T and $\overline{\delta_{>T_{min}}}$ = mean isotope value for all samples in the subset extracted for longer than T_{min}.

RESULTS AND DISCUSSION

The extraction timing curves (Figs. 2–4) showed a common pattern. The isotopic value of extracted water increased with extraction time until a certain threshold, after which the isotopic value of extracted water remained essentially constant regardless of further increases in extraction time. The time at which this threshold was initially reached was the minimum extraction time required to obtain an isotopically unfractionated water sample (T_{min}). T_{min} varied depending on the source material (Table 1). Woody stems required the longest extraction times (60–75 min), with shorter times for clay soils (40 min), sandy soils (30 min), and leaves (20–30 min).

Table 1. Minimum extraction times (T_{min}) required for an unfractionated water isotope value for all materials sampled in this study. Range and standard deviation (SD) of $\delta^{2}H$ and $\delta^{18}O$ were calculated for all samples extracted for T_{min} and longer

	T_{min}	Range in δ ($\geq T_{min}$)		SD of δ ($\geq T_{min}$)	
Material	(min)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)	$\delta^{2}H$ (‰)	$\delta^{18}O$ (‰)
A. altissima stems	75	1.44	0.67	0.49	0.21
P. edulis stems	60	11.9	1.22	3.30	0.28
J. osteosperma stems	60	9.38	1.27	2.58	0.33
Clay soil	40	1.31	0.22	0.54	0.08
Sandy soil	30	2.52	0.37	0.69	0.15
M. grandiflora leaves	30	7.27	2.16	2.94	0.81
P. nigra needles	30	2.31	1.20	1.04	0.50
A. altissima leaves	20	4.90	1.27	1.56	0.39

In line with the findings of Araguas-Araguas *et al.*,[17] our data suggest that extractions do not have to reach completion (100% recovery of water) to obtain an essentially unfractionated water sample. Once T_{min} was reached, only a very small amount of water remained in the sample; however, it took up to an additional 3 h to recover this remaining fraction of water (data not shown). For the purposes of obtaining an unfractionated isotope value, within our current analytical precision, this additional extraction time appears unnecessary.

For woody stems, T_{min} varied between 60 and 75 min for the three species tested (Fig. 2). The angiosperm (*A. altissima*) had the longest T_{min} of 75 min. The two conifer species (*P. edulis* and *J. osteosperma*) had a shorter T_{min} of 60 min. For leaves, T_{min} varied between 20 and 30 min for the three species tested (Fig. 3). *A. altissima* had the shortest T_{min} of 20 min. *M. grandiflora* and *P. nigra* had a longer T_{min} of 30 min.

The variation in the extraction times for the stems and leaves may well be due to variation in the morphology of the material in question. For stems, pore-size between vessels (angiosperms) and tracheids (conifers), or the wood density of the species in question, may influence T_{min}. For leaf material, the variation in T_{min} may be due to leaf toughness or specific leaf mass. We were not able to systematically test these hypotheses in our study and they remain questions that could be addressed by further research.

For several of the materials sampled, the variance in the post-T_{min} δ values was greater than the precision of the analytical method (Table 1). Both sandy and clay soils showed particularly low variance and standard deviation of extracted water (Table 1). Previous studies have indicated that extracting water from clay soils can be problematic due to interactions between pore water and weakly bound water in the clay matrix.[7,16,17] This did not appear to present a problem for obtaining a clean extraction timing curve in this study.

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CONCLUSIONS

In this paper we have provided estimates of minimum extraction times and examined variation in isotopic ratios of water extracted from plants and soils. We estimated minimum extraction times to be 60 to 75 min for woody stems, 40 min for clay soils, 30 min for sandy soils, and 20 to 30 min for leaves. We acknowledge that these estimates could vary for different extraction systems and different materials.

Our results suggest that some gains in sample throughput efficiency are possible by reducing extraction time to T_{min}. We suggest that the approach presented in this paper could be of use to others seeking to optimize extraction efficiency for different materials or different extraction apparatuses. Additional gains in throughput are likely to come from the careful design of extraction apparatuses allowing the simultaneous extraction of multiple samples. Ultimately, to remove the bottleneck of water extraction, a new technological advance that eliminates the need for water extraction is required. Such a method exists for leaf water analysis,[20] but not at present for other materials. Advances in this direction would greatly facilitate the use of water isotopes in ecological studies.

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