Ecohydrological separation in wet, low energy northern environments? A preliminary assessment using different soil water extraction techniques

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Ecohydrological separation in wet, low energy northern environments? A preliminary assessment using different soil water extraction techniques

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Abstract

Ecohydrological studies in seasonally dry climatic regions have revealed isotopic separation of the sources of water used by trees and those that generate stream flow, also referred to as the 'two water worlds' hypothesis. Here we investigated whether similar separation occurs in a wet, low energy northern (Latitude 57^{o}) environment in Scotland. For two common soil types (Histosols and Podzols) at three soil depths, and at both forested (with Scots Pine (*Pinus sylvestris*)) and non-forested sites, we compared the stable isotope composition of soil water held at increasing soil water tensions. These were assessed by different soil water extraction techniques: Rhizon samplers (mobile water), centrifugation at different speeds (representing different tensions), and cryogenic extraction (bulk water). Sampling occurred during a relatively dry summer. Water that was held at increasing tensions appeared more depleted than more mobile water, consistent with older (winter) precipitation. This pattern was independent of soil type, vegetation cover, and time during the growing season, although there was a slight tendency towards less separation with soil depth. Nevertheless, soil waters in this generally wet, low energy environment exhibited

only minor evaporative enrichment, limited to the upper soil profile only. Furthermore, stream water showed no deviation from the local meteoric water line. Preliminary sampling for tree xylem water suggested uptake of evaporated soil water from the near surface soil horizons (upper 10 cm) where fine root densities are concentrated. For Histosols in particular, tree water appeared lagged in its isotopic composition compared to the soil water time series. Although more work is needed to fully test the 'two water worlds' hypothesis, our initial analyses did not provide clear evidence to support this in wet, low energy northern environments.

Keywords: isotopes; water storage; soil water extraction; tree water use

1. Introduction

Catchment water storage, mixing and flux processes usually have a strong influence on stream flow generation and solute transport (Kirchner *et al*., 2000; 2001; McDonnell *et al*., 2010; Rinaldo *et al*., 2011). It has often been assumed that water in subsurface stores is well mixed and that vegetation assimilates water that would have otherwise contributed to groundwater recharge and stream flow. These assumptions are well embedded in most (eco)hydrological models and conceptual frameworks. However, recent work has challenged this by suggesting that at least in seasonally warm and dry climatic regimes, vegetation uses tightly bound water which is isotopically distinct from more mobile water that contributes to streamflow (e.g. Brooks *et al*., 2010, Goldsmith *et al*., 2012). As such, these studies have speculated on the coexistence of two pools of water in the subsurface that do not mix, not even during or after consecutive precipitation events. This 'two water worlds' hypothesis (McDonnell, 2014) is supported by stable isotope (δD and $\delta^{18}O$) tracer analyses of precipitation, soil, stream, and xylem water samples. Stable isotopes are useful tools to gain insights into hydrological processes (Kendall and McDonnell, 1998; Vitvar *et al*., 2005) such as subsurface water storage, mixing and transport processes (e.g. Gazis and Feng, 2004; Mueller *et al.,* 2014; Tetzlaff *et al*., 2014; Birkel and Soulsby, this volume), and also plant water use (e.g. Dawson and Ehleringer, 1991; Brandes *et al*., 2007; Bertrand *et al*., 2014). In the context of the 'two water worlds' hypothesis, the term 'ecohydrological separation' has been used to describe instances where the vegetation is using water of a different (isotopic) character than that found draining freely through the soil profile and into the stream. In other words, the vegetation has been shown to preferentially draw water from a particular source, where several co-exist. Previous work (e.g. Brooks *et al*., 2010, Goldsmith *et al*., 2012) demonstrated a clear distinction between tightly bound water that has the signature of strong evaporative enrichment and which is used by the vegetation on the one hand, and more mobile waters with less enrichment and which recharges the stream on the other.

It is well known that the chemical composition of extracted soil water may depend on the extraction techniques used (e.g. Walker *et al*., 1994). This is related mostly to differences in the matric tension exerted in the extraction so that water is drawn from different pore size distributions (Tiensing *et al*., 2001). Secondary effects include, for example, soil type (Aragúas-Aragúas *et al*., 1995), and extraction temperatures (Ingraham and Shadel, 1992; Walker *et al*., 1994). Traditional soil water extraction methods for stable isotope analyses include lysimeter porous cups or samplers in the field, or soil core sampling for laboratory based cryogenic vacuum distillation, azeotropic distillation, centrifugation, and mechanical squeezing (see reviews by e.g. Barnes and Turner, 1998; Soderberg *et al*., 2012). The key difference between these field *versus* lab techniques is that porous cups extract water that is held at low tensions (i.e. 'mobile' water at tensions less than 200 kPa), while most laboratory methods extract all water (down to 10-15 MPa) so that their chemical composition represents the bulk water (including more 'tightly bound' water) held by the soil (Landon *et al*., 1999; Figuéroa-Johnson *et al*., 2007; Zhao *et al.*, 2013). In this context, different techniques can therefore be used to extract water that is held at different soil water tensions (i.e. in pores with increasingly smaller sizes). Several studies have demonstrated a clear distinction between the isotopic composition of mobile water extracted *via* lysimeters and bulk water extracted *via* cryogenic vacuum distillation (e.g. Brooks *et al*. (2010) in the USA, Goldsmith *et al*. (2012) in Mexico, and Zhao *et al.* (2013) in China).

The mechanisms that control subsurface isotopic separation in catchments are poorly understood (McDonnell, 2014). In relatively dry mineral soils with high clay content and consequently high cation exchange capacity, clay particles can interact with soil water to create "pools" of different waters with varying isotope compositions (Aragúas-Aragúas *et al*., 1995; Meißner *et al*., 2013; Oerter *et al*., 2014). However, a main factor controlling soil water separation may be a strong seasonality in climate (McDonnell, 2014); where considerable soil drying might be required to allow for (evaporated) precipitation inputs to enter and be retained by the smaller pore spaces for prolonged periods of time (Brooks *et al*., 2010; Goldsmith *et al*., 2012). When soils are dry and precipitation intensity and duration are low, Tang and Feng (2001) showed that new water could not effectively replace old water in a soil column. They argued that traces of old water could be present in the soil matrix for a long time or even throughout the entire growing season. However, it should again be stressed that all studies that specifically explored the occurrence of separation based on isotopic signatures have been conducted at sites with strongly seasonal patterns in precipitation and high evaporative losses. To further understand the wider relevance of these isotopic implications for soil water storage and flow partitioning, there is a pressing need to explore contrasting environments across different hydroclimates (in particular less seasonal in precipitation inputs) and in different soil types (McDonnell, 2014; Tetzlaff *et al*., this volume).

In this paper we explore soil water storage and evidence for ecohydrological separation in a low energy, wet northern catchment. In these humid, boreal environments, there is usually limited seasonality in precipitation inputs, energy for evapotranspiration is relatively low, and soil water storage is persistently high (Tetzlaff *et al*., 2014; Geris *et al*., 2015a), particularly when compared to the more seasonal and drier climates in previous studies. We hypothesise that in such wetter environments, soil water stored in smaller pores is more likely to exchange or mix with more mobile water. We investigated this hypothesis for various soil-vegetation units in the eastern Scottish Highlands, UK, by comparing the stable isotope composition of soil water held at different tensions by using various extraction techniques: porous Rhizon samplers (inferred matric tension of < 200 kPa), centrifugation (inferred tension of ~200, ~700, and ~1100 kPa) and cryogenic extraction (up to 15 MPa). We compared soil water that was sampled during a growing season in a 10 year return period drought (Geris *et al*., 2015b), assuming that if separation of soil waters would exist, it would be most marked during such a period with relatively high evapotranspiration. Our specific questions were:

(1) Can any different pools of soil water in northern environments be identified by comparing the stable isotope composition of soil water held at different tensions?

(2) If so, how might differences in soil type affect these patterns?

(3) How does the water use of vegetation, as identified by the xylem isotope composition, compare to the measured soil water isotope composition of water under different tensions?

2. Study area

This study was carried out in the Bruntland Burn (3.2 km^2) experimental catchment in the Cairngorms National Park, Northern Scotland, UK (Figure 1) where forests are part of the boreal forest biome of the upper latitudes of the Northern hemisphere. The prevailing wet climate has fairly cool summers (June average temperature = 15.8 ^oC), cold winters (February average temperature = -0.7 ^oC), and 1100 mm of annual precipitation which is relatively evenly distributed throughout the year. Annual runoff (700 mm) greatly exceeds potential evapotranspiration estimates (400 mm). Elevations range from 248 to 539 m a.s.l. (mean 351 m a.s.l.) and the mean slope is ~13°. The catchment is predominantly underlain by metamorphic (54 %) and granite (46 %) bedrock. The geology gives rise to low base status soils and the landscape is characterised by a strong glacial legacy. The widened valley bottom has thick glacial drift deposits up to 40 m deep (Birkel *et al*., 2015). These are covered by thick (>1 m) waterlogged peat soils in the valley bottom, which gradually thins to shallow peats (0.5 m) on the lower hillslopes. These Histosols cover 21% of the catchment. The dominant soil types on the hillslopes are humus-iron Podzols (Spodosols, 36% of the catchment area), which thin to Leptosols (Entisols, 14%) and bedrock outcrops (29%) at slopes >25°. The Podzols have a ~0.2 m O- horizon that overlies the mineral sub-soil.

The hydropedology has an important control on the spatial distribution of water storage and fluxes. The poorly draining Histosols in the riparian zone are permanently wet and have high total water storage. Hence, the dynamic storage differences are small and runoff generation is predominantly through near surface runoff flow pathways. The Podzols on the hillslopes experience distinct wetting and drying cycles that coincide with precipitation events (Tetzlaff *et al*., 2014). In these more freely draining soils, the dynamic storage changes are larger and there is more recharge to deeper flow pathways (Birkel *et al*., 2015). The spatial distribution of vegetation shows a strong connection with soil type. The Histosols are characterised by *Sphagnum spp*, *Molina caerulea* and *Myrica gale* dominated peat bogs. The most widespread vegetation on the hillslopes is heather (*Calluna* and *Erica* species) vegetation. Trees, mainly native Scots Pine (*Pinus sylvestris*), are able to grow on all main soil types, but after centuries of widespread deforestration their distribution is now limited to steeper areas (20% of the catchment) generally inaccessible to deer grazing. During relatively dry conditions, there is some indication that tree cover can affect hydrological responses, e.g. through increasing evapotranspiration and exacerbating soil water storage drawdown trends (Geris *et al.,* 2015a; 2015b), although these effects are small at the catchment scale. Tracer studies have indicated that, in general, stream flow has a strong connection to soil water stored in the Histosols and that the riparian zone contributes the majority of water in the stream (Tetzlaff *et al*., 2014). Direct groundwater contributions (as a separate source compared to soil water contributions) are in the order of 25-35% of annual stream flow (Soulsby *et al*., 2007).

3. Methods

To examine soil water storage and flow separation in low energy, wet environments, we compared hydrometric data and stable isotope composition of precipitation, stream water, xylem water, and different soil waters for four soil-vegetation units in the Bruntland Burn catchment (Figure 1). The main study period reported here spanned the growing season of 2013 (May – August), though basic hydrometric and isotope monitoring of precipitation and stream flow is part of a longer term study. As April still experienced significant snow cover on the ground (Geris *et al*., 2015a), the start of the 2013 growing season was defined at the beginning of May. We focussed on the summer growing season, as long residence times of water in the trees can decouple isotopic signals of xylem and soil source water in winter (Brandes *et al*., 2007). The four soil-vegetation units are broadly representative of the dominant soil and vegetation units in such boreal environments and include two Histosol sites with Sphagnum (Hs) and Scots pine forest (Hf) cover, and two Podzol sites with Heather (Ph) and Scots pine forest (Pf) cover, respectively (Figure 1). For a detailed description of these four sites and the soil physical characteristics, the reader is referred to Geris *et al*., 2015a.

Hydrometric data included daily catchment precipitation and stream flow. Further climatological data were available from a nearby automatic meteorological station located 1 km west of the Bruntland Burn. These were used to estimate daily potential evapotranspiration rates using a simplified version of the Penman-Monteith Equation (cf. Dunn and Mackay, 1995). Furthermore, at the four study sites, 15 min soil moisture data were collected at three different monitoring depths (10, 30, and 50 cm), which largely coincide with the main soil horizons (*Geris et al*., 2015a). At each of these monitoring depths, volumetric soil moisture (VSM) content was measured with *Campbell Scientific* 650-VS time domain reflectometry (TDR) probes. Average values of two replicate probes are presented here. VSM data were collected for each of the three depths for the Podzol sites. For the Histosol sites, VSM was attained at 0.1 m depth only, as the lower soil layers (0.2 m and beyond) were permanently saturated.

During the full duration of the study period, daily precipitation and stream water samples were collected via *ISCO* automatic water samples for stable isotope analyses. In addition, fortnightly soil water samples were collected via *Rhizosphere Research Products* MacroRhizon moisture samplers to assess the dynamics of mobile water. These are small porous Rhizon samplers (Di Bonito *et al*., 2008) that sample water under vacuum. Vacuum was obtained via 30mL syringes with retainers to hold the vacuum. After initial flushing of the system, the vacuum was held for 10-30 minutes until at least 10mL of soil water was collected. The vacuum is assumed to have tension strengths smaller than 200 kPa. Two hundred kPa is the bubble point of the Rhizon samplers and it also represents the lowest inferred tension exerted using any of the other extraction methods used in this study.

In the middle of the growing period when conditions were unusually dry, two more extensive sampling campaigns (Table 1) were carried out where soil water was further extracted using other techniques. These represent water that is held at a range of soil water tensions (Table 2). In addition to the Rhizon mobile water collection, the second method involved centrifugation of intact soil cores at various speeds that represent different matric tensions. During the two extensive sampling campaigns, 12 soil cores from each of the three horizons at each site were extracted and transferred into centrifuge tubes (each 2 mL max volume) with 0.45µm pore size filters. The filtration unit was embedded within the centrifuge tube. These tubes were immediately sealed with parafilm and refrigerated until analyses. A temperature controlled (5 ^{o}C) *Thermo Scientific* Fresco 21 Microcentrifuge was used to avoid isotopic fractionation effects as a result of the centrifugation. Samples were split into three groups (n = 4 for each group) and spun for 1 hr at different speeds to represent water that is held at inferred matric tensions up to ~200, ~700, and ~1100 kPa. Initial tests showed that centrifugation times of 1 hr were sufficient, after which no more water would be extracted at a particular speed. Relative centrifugal force was converted to soil water tensions using transformations based on simple soil physics (Edmunds and Bath, 1976). Samples with water extracted up to ~200 kPa were spun again at a speed representing ~1100 kPa to sample water held between ~ 200 and 1100 kPa.

The third extraction method involved cryogenic vacuum distillation. Two soil samples of each horizon were collected in 40 mL vials, sealed with parafilm, and frozen until analyses. Cryogenic extraction was carried out in the Surface Chemistry and Catalysis laboratory, University of Aberdeen, following the procedure of West *et al*. (2006). Due to relatively high water content, extraction times were long (>2 hr), which limited the analyses to 2 samples for each horizon and for the upper two soil depths only. After extraction, samples were oven dried (105°C) for 24 hrs. Pre and post drying weight was compared to determine extraction efficiency. Full extraction efficiency (no difference in pre and post drying weight) was achieved for all samples.

For the forested sites, xylem samples were collected from three mature trees using an increment borer during the two more extensive soil sampling campaigns. Trees were selected based on their vicinity to the soil water sampling site (Figure 1). Storage and cryogenic extraction followed the same procedure as the soil samples. One xylem water sample for the Podzolic soil did not achieve full extraction efficiency. The stable isotope analysis result of this sample was therefore excluded from the analyses here.

Precipitation, stream and soil water samples were analysed for stable isotope composition with a *Los Gatos* DLT-100 laser liquid water isotope analyser following standard protocols. Data are provided in the δ-notation (‰) relative to the Vienna Standard Mean Ocean Water (VSMOW) and the precision of measurements is ±0.6‰ for δD and ±0.1‰ for δ^{18}O. It is known that water extracted from both soil and vegetation samples may contain organic contaminants which can interfere with the laser isotope ratio spectroscopy (West *et al.*, 2010). This is typically strong for vegetation samples (e.g. Zhao *et al.*, 2011), while often minor for soil water samples (Schultz *et al.*, 2011). *Los Gatos* software was used to identify any sample contamination (cf. Schultz *et al.*, 2011; West *et al.*, 2011). For the samples in this study, we found that only vegetation samples were contaminated by small organic components. We therefore treated these with activated charcoal to reduce the concentration of organics from the water samples (c.f. West *et al.*, 2010) and analysed them using traditional isotope ratio mass spectrometry, to avoid laser contamination effects. These samples were analysed at the Scottish Universities Environmental Research Centre (SUERC) Mass Spectrometry Facility Laboratory in East Kilbride. The precision of these stable isotope measurements is ±2.0‰ for δD and ±0.5‰ for $\delta^{18}O$.

To identify differences between different soil and xylem waters across the four monitoring sites, their isotope signatures were compared against their location with respect to the global and local meteoric water line. This was also used to test for any evaporative fractionation effects in any of the source waters. Furthermore, the results of the two extensive field sampling campaigns were evaluated within the longer term context of precipitation, stream and mobile soil water time series.

4. Results

4.1 Hydroclimatological conditions

The study period included an unusually dry and warm summer with relatively little precipitation, high evapotranspiration and low discharge rates (Figure 2). Compared to long term hydroclimatological averages, similar dry periods have an estimated return period of 1 in 10 years (NHMP, September 2013; Geris *et al.*, 2015b). When analysed for the total study period, precipitation inputs (240 mm) were low compared to stream discharge (120 mm) and potential evapotranspiration (333 mm) outputs, resulting in a considerable decline in catchment average storage. Figure 2 shows that there were two distinct periods with no precipitation (9 days in early June and 18 days during early to late July). This is atypical for the eastern Scottish Highlands which are generally characterised by year-round low intensity but frequent precipitation. The isotope composition of precipitation inputs during the study period was enriched (weighted δD mean = -52.0 ‰; standard deviation = 17.7 ‰) in comparison to long term averages (weighted δD mean = -59.1 ‰; standard deviation =

21.1 ‰), but in line with expected seasonality. Stream water isotope signatures demonstrate considerable damping, suggesting significant mixing of event and old precipitation water before it enters the stream.

Table 1 shows the hydroclimatological conditions during the two extensive soil and tree xylem sampling campaigns on June 11th and July 22nd. These were specifically targeted to coincide with the end of the two periods with low precipitation. Although the conditions were relatively dry during both days, July 22nd experienced higher air temperatures and potential evapotranspiration rates than June 11th. In addition, the second day was later in the growing season and the catchment was generally drier (Table 1; Figures 2-3).

4.2 Soil water storage

Considering the dry summer 2013 conditions, the dynamic soil water storage changes in the Histosols were relatively small (Figure 3). Compared to the Histosols, the Podzols experienced stronger drying. This resulted in reduced extractable water availability for sampling, particularly in July. In general, the storage changes in the upper soil profiles were most marked, and also more variable for the Podzols than in the Histosols. However, as a result of the two exceptionally dry periods, the VSM content during the extensive sampling days was, although overall still high, relatively dry for these four specific soil-vegetation units, mainly in July (see also Figure 3). In particular for the tree cover sites, the upper soil horizons (as measured at the 0.1 m depth) VSM readings were as low as 0.47 for the Histosol (Hf) and 0.16 for the Podzol (Pf) during the July sampling campaign.

The soil water isotope time series in mobile waters extracted from lysimeters showed that for most soil horizons, but particularly the surface horizons, there was a general shift towards more enriched samples up until the end of July, after which more depleted samples were observed (Figure 3). This can be directly related to a high input of relatively depleted precipitation at the end of July and beginning of August (Figure 2). Overall, there was more variability in the isotope signatures of the drier, freely draining Podzols in response to precipitation inputs than in the wetter Histosols. The mobile water at all sites was most enriched during the two extensive sampling campaigns, in the context of the study period timeseries at a particular site (Figure 3). This was not the case for the Hf site, where the isotopic composition of the July sample was slightly more depleted than the earlier summer samples, although these differences were small.

There are four main observations on the soil water isotope signatures from the different extraction techniques. Firstly, overall, water that was held at higher inferred tensions was more depleted than the more mobile water (Table 2; Figures 4-5), suggesting some degree of separation. This was observed when comparing the results from the three extraction techniques (porous Rhizon samplers > centrifugation > cryogenic extraction). However, there was no clear difference between water samples obtained by extraction at different centrifugation speeds. In addition, the analyses indicated that separation was strongest in the upper soil profile at 0.1 m.

Secondly, the patterns observed are consistent for all four soil-vegetation units, with the caveat that the relatively dry conditions of the Podzols, and the rather small soil core sampling size, limited the water that could be extracted via the centrifugation method. Nevertheless, the overall patterns are similar across the four sites, even in the absence of centrifugation data. This suggests that any isotopic separation appeared to be independent of soil type as well as vegetation cover. However, in general the Podzolic soil waters were more enriched than those of the Histosols and therefore reflected more recent precipitation inputs. This observation could be related simply to the more freely draining nature of the Podzols, with lower moisture content available for mixing and shorter residence times in general.

Thirdly, Figure 5 demonstrates that the June and July sampling trips both showed similar separation patterns. Moreover, there was no clear difference between specific samples (i.e. the July samples are in general not more or less enriched than the June samples). This may imply that the separation was consistent during the monitoring period and provides a justification for bulking of June and July samples in all other Figures and Tables.

Finally, there is very limited evidence to suggest strong evaporative fractionation of any of the soil water samples. Although mobile waters sampled with the Rhizon samplers were more enriched than the more tightly bound waters, there was little to no major deviation from the meteoric water line at most sites (Figure 5). The strongest indication of such fractionation effects is evident for the forested Podzol (Pf) site, in the upper soil profile at 0.1 m depth in the O Horizon.

4.3 Water stores and vegetation water sources

Like the previous plots, Figure 6 demonstrates that there is a vertical profile in the isotope signatures of the mobile soil waters for all sites, showing the most depleted waters at greater depths. There is also a strong overlap between the soil water, in particular for the Histosols in the riparian zone, and stream water as indicated by their respective isotope compositions. Figure 6 shows the soil waters of forested sites only, but data (not shown here) demonstrated similar overlaps for the non-forested sites (see also Tetzlaff *et al*., 2014; Geris *et al*., 2015a). Both summer (May-August 2013) and the previous winter (November 2012-April 2013, from Geris *et al*., 2015a) mobile soil data were included to assess potential tree water sources and account for any delay in water uptake. The summer soil water data also include the results from the centrifuged and cryogenically extracted samples. Overall, all of these summer soil water data largely plot in the same space as the stream data (Figure 6). This indicates there is no strong evidence to suggest that there is a marked isotopic separation between the water stored in the soils available for vegetation uptake and that reaching the stream.

The space in which the isotope composition of Scots Pine xylem waters plotted was similar to the soil and stream water, but different for the two soil types. Xylem samples in cores taken from trees growing on the Histosol site (Hf) generally plotted slightly below the meteoric water line and in a different space from stream water as well as summer soil waters sampled there (Figure 6), though the depleted deuterium values were more similar to winter soil waters (open symbols in Figure 6). They also did not plot directly on the evaporative line of any of the soil waters. For the trees growing on the Podzolic soil (Pf), most xylem samples plotted directly on the meteoric water line and within the space of both soil and stream water. However, one sample showed some evaporative fractionation effects and plotted in the space of soil water samples that were extracted using centrifugation and cryogenic extraction (i.e. potentially representing water held at higher tensions) from the upper profile.

5 Discussion

5.1 Soil water storage and flow partitioning for different soil types

The results of the various extraction techniques indicated that water held in the smaller pores was generally more depleted than the mobile soil water. Although a different climate regime and set of soil types, these results are consistent with previous studies using different extraction techniques to determine soil water isotopes (e.g. Landon *et al*., 1999; Figuéroa-Johnson *et al*. 2007; Brooks *et al*., 2010). The results could suggest that for all four soil-vegetation units, and for a prolonged period of time (> 1 month), the mass transfer between mobile and less mobile subsurface pores was low, as indicated by the isotopically distinct soil water pools. The more depleted bulk water samples appeared to reflect older winter precipitation inputs and be less affected by summer evaporation. Consistent with basic soil physics, this suggested that the water held at high tensions is older than that held at lower tensions, and it is less mobile. However, although the soils in this study experienced drying, the moisture content remained relatively high throughout the study period, apart from in the forested Podzol. In particular during the June sampling campaign, VSM data were still comparable to conditions during wetter winter periods at most sites (see Tetzlaff *et al*., 2014). Several previous laboratory and field investigations have shown that particularly at lower water contents, soils have greater fractions of immobile water and slower mass transfer between the mobile and immobile regions (Padilla *et al*., 1999; Tang and Feng, 2001; Zhao *et al*., 2013). Our results suggest that in humid, low energy environments, similar effects are evident even when soils still remain relatively wet. The difference is that our study was set in an energy limited system, rather than a water limited system as reported elsewhere.

It has recently been identified that soil chemical properties (in particular high clay cation exchange capacities, CEC) could also cause isotopic separation in soil waters (Aragúas-Aragúas *et al*., 1995; Meißner *et al*., 2013; Oerter *et al*., 2014). Although we do not have data on cation exchange capacity and clay content, the soil parent material is generally glacial drift comprised of silty-sand and larger clasts. The clay content is usually low in mineral horizons and very low in organic horizons, where cation exchange sites will be on organic substances. At three nearby sites with similar soils, Barton *et al*. (1999) found CEC values in the order of 1-5 mmol/kg. Furthermore, the study by Oerter *et al*. (2014) indicated that such cation exchange capacity effects were only strong for VSM on the order of 0.05 and significantly decreased at 0.30. In this context, water content in this study was relatively high, in particular for the June sampling campaign, and clay content was generally low. Hence, this would suggest that any soil property effects related to clay content cation exchange were small. This is further supported by similar separation occurring across the different soil-vegetation units. However, we recognise that, in order to fully understand the mechanisms that drive soil water storage and flow separation at our site and at any other, further testing that considers pH, temperature, microbiological activity and other factors that could potentially affect the isotope composition of soil water would be required.

The isotopic differences between the three extraction techniques were relatively small. The maximum difference in δD between mobile and bulk water was on the order of 10‰, and often less (Table 2). This is less than half the δD range observed annually in highly damped stream water (around 25‰, varying between extremes of -75.2‰ to-49.6‰, see Geris *et al*., 2015a). In addition, the 10‰ difference was observed only between extraction techniques that sampled water held at tensions to the most extreme, while little to no differences were found between water extracted at the different centrifuge rotations. Furthermore, the depletion in the bulk soil water was also still far less than the observed range of precipitation in the previous winter, which is up to -142.9‰ (Geris *et al*., 2015a). Multifactorial experiments with suitable statistical analyses could establish the significance of actual differences between the results from different extraction techniques and soil water depths. However, the limited data collected here are too small a sample size to meet the underlying criteria needed for rigorous statistical testing. Although the results here do indicate certain patterns as described above, further sampling would be required to fully confirm or refute differences between soil water sampling techniques.

Apart from the upper soil profile of Pf, we did not find strong evaporative fractionation in the bulk soil water, contrary to what was observed in earlier studies by Brooks *et al.* (2010) and Goldsmith *et al*. (2012). One explanation is that soil evaporation is much more limited in energy-limited hydroclimatic regimes where soils remain wet (Barnes and Turner, 1998). Previous work at the same site over a 2 year period less affected by drought than in the current study also found very limited evaporative fractionation effects in mobile soil water and only under the driest conditions (Geris *et al.*, 2015a). An alternative explanation is that such fractionation in the bulk water has a limited effect on the isotopic composition as the soil water content is relatively high and only a small proportion of the total water which is held in the larger, more aerated pores in the upper profile, is affected. However, in this case we might have expected to find fractionation effects in the soil water samples that were centrifuged twice. Our results did not indicate that this was the case. In general though, our comparative results agree with other studies that found less difference between mobile and more tightly bound water for wetter soils and at greater depths (e.g. Zhao *et al*., 2013).

5.2 Vegetation water use

When compared with the isotopic composition of potential water sources, xylem water can provide insights into the water assimilated by trees. Assuming no fractionation at the time of uptake or during transport within the trees (Ehrlinger and Dawson, 1992), plant xylem water should have the same isotope ratios as subsurface water which will ultimately reach stream networks, assuming complete mixing of subsurface water occurs. However, the studies by Brooks et al. (2010) and Goldsmith et al. (2012) showed strong isotopic separation between xylem water on the one hand and mobile and stream water on the other, which was consistent throughout the season. In addition, they found that the xylem water also indicated strong fractionation effects from evaporative processes and mostly resembled bulk water that was extracted via cryogenic extraction. This suggested that tree water uptake was preferentially sourced from tightly bound subsurface soil water. Earlier work by Dawson and Ehleringer (1991) also demonstrated that riparian trees did not use water from the stream but from deeper groundwater sources, although their study was limited by the use of $\delta^{18}O$ alone. Only when both stable isotopes are compared against the meteoric water line, can different water stores and evaporative enrichment be explored in greater depth.

Our preliminary vegetation water analyses showed that most samples plotted in the same meteoric water line space as the soil water samples, although others not. There were contrasting results between different soil-vegetation units and also within one unit (notably the forested Podzol site, Pf). However, in all cases, the space in which the xylem isotope composition data plotted was different in comparison to results of earlier studies, which showed a 'below the meteoric water line' grouping between bulk soil waters and xylem waters (Brooks *et al*., 2010; Goldsmith *et al*., 2012). We did not find strong connections between these two, neither between xylem water and any of the sampled soil or stream water.

There are multiple working hypotheses to explain the observed xylem water data. For the Podzol site, most xylem samples did actually plot on the meteoric water line (MWL) and in the same MWL space as soil and stream water. These data could indicate that trees do not use water from a distinct subsurface water pool and hence, the lack of evidence for isotopic separation at this site. However, one or two samples do show some effects of evaporative fractionation and plot in a similar MWL space to the more tightly bound water in the upper 10 cm of the soil profile. Several studies across northern environments have demonstrated that for Scots Pine most of the fine root production and turnover is in the humus layer (i.e. upper few cm) and upper 0.1 m (Roberts, 1976; Persson, 1980; Bishop and Dambrine, 1995; Čermák *et al*. 2008). This is above the uppermost sampling depth, though this is also where evaporation is highest (as hinted in the July Pf 0.1 m isotope samples). As the soils never dried out during the study period, moisture in the 0 – 0.1 m depth was still likely to be plant available, but also likely to have been fractionated due to soil surface evaporation. Moreover, the soil atmosphere would have been increasingly aerated as the soil dried, which may have allowed the soil water to start to re-equilibrate with the air as well. Thus, a possible hypothesis for the evaporated signal in some plant water samples is that the upper 0-10 cm could be the source of the majority of tree water in summer when the xylem samples were collected. However, this does not imply that the trees preferentially take up water from either tightly bound or mobile water, as was previously argued in the context of the 'two water worlds' hypothesis. Although sampling tree root distributions was beyond the scope of this work, the location of the majority of fine roots in the upper 10 cm of the soil is consistent with the literature on Scots Pine (Roberts, 1976; Persson, 1980; Bishop and Dambrine, 1995; Čermák *et al*. 2008).

While this hypothesis would be the most likely explanation for the evaporated xylem waters of the trees at the Podzol site, the xylem data for the trees on Histosols largely do not lie within any evaporative MWL of near surface soil water as observed during the study period.

However, highly depleted winter isotopes were observed at site Ht (Geris *et al.*, 2015a; Figure 6), from which the Ht xylem water could have been sourced, although also subject to some evaporative fractionation. In addition to the processes described above, this might imply that the apparent slight separation between xylem and soil/stream water could have been exacerbated by slower tree water uptake influencing its isotopic composition compared to the soil water. Long residence times of water transport in trees, in combination with suppressed transpiration during and just after rainfall events could cause such delays (Brandes *et al.*, 2007; Penna *et al.*, 2013). Scots Pine growth rates in Scotland are low, especially on acidic Histosols which are waterlogged and nutrient poor. Sapflow values in the literature suggest that water movement in Scots Pines is relatively slow in the order of 1 to 6 mm d^{-1} (Köstner *et al.*, 1996; Brandes *et al.*, 2007). Moreover, a very cold spring preceded the warm, dry summer with temperatures well below average well into May which could have delayed the onset of transpiration in this particular year.

Another hypothesis to explain evaporated plant water signals could be related to internal physiological processes within the tree. Several field and laboratory studies have demonstrated that no isotopic fractionation takes place at the time of uptake or during transport within the trees until the water reaches the leaves where transpiration from the leaf surface causes isotopic enrichment of leaf water (Ehrlinger and Dawson, 1992; Brunel *et al.*, 1994). However, a few studies have demonstrated some isotopic enrichment of xylem water that could be linked with either stem cuticular evaporation (Dawson and Ehleringer, 1993), although this is most common for young deciduous trees, and/or xylem-phloem exchange (Cernusak et al., 2005; Bertrand *et al.*, 2014), and only under water stress. Neither possibility can be ruled out, but given the soil moisture content, the mature Scots Pine trees in this study were unlikely to have experienced severe water stress.

Finally, we recognise that there are relatively limited numbers of xylem samples in this study and clearly more data are needed to fully understand the interactions between water use, fractionation effects, and the apparent isotopic separation of soil water for some sites. Moreover, because of the large potential variability between trees within and between sites, a greater sample size is needed to characterise this heterogeneity. Although we aimed to sample trees of similar age, diameter and height, subtle differences between these tree characteristics are known to cause additional natural variability in xylem isotope data between trees, and for this reason sample sizes of at least one order of magnitude larger than deployed here might be advised (Goldsmith *et al*., 2012).

5.3 Implications and future directions

Although the exact mechanisms remain unclear and are likely to be different from those in drier and more seasonal climates, this study has shown that soil waters in wet, low energy northern catchments extracted under different tensions exhibit some minor isotopic differences. This could be associated with variations in fractions of immobile water and mass transfer between mobile and immobile regions. However, overall, there was no marked separation from the stream water or the local meteoric water line, as has been reported previously. Furthermore, Scots Pine xylem water isotope data largely demonstrated that there was minimal difference between the tree, soil, and stream water, although some xylem samples suggested water uptake from evaporated near surface soil water. This study has not provided evidence that ecohydrological separation, consistent with the 'two water worlds' hypothesis occurs in wet, northern climates and further work is needed to explore the exact mechanisms that drive such processes. In addition, this study focussed specifically on a relatively dry period, assuming that if separate water pools' in the soil and vegetation water would exist, it would be most extreme under these conditions. In comparison to the previous studies in wet Mediterranean climates, the isotopic separation found here was relatively small and did not show any effects of evaporative fractionation despite the relatively high local evapotranspiration rates. As the study period experienced an exceptionally dry growing season, it seems improbable that much stronger evaporative fractionation processes than those observed here are common at this site. It is therefore unlikely that more marked isotopic separation in support of the 'two water worlds' hypothesis would occur during wetter summers.

However, repeated sampling in wetter and colder conditions should be used to further test hypotheses regarding the extent and duration of the separation between mobile and bulk water as observed between June and July 2013. This is one of the first studies that specifically investigated such soil water storage and isotope separation in highly organic soils in higher latitude regions. As such, this study provides an important first step towards a better understanding of subsurface storage and transport, as well as tree water use. In particular, we showed that sampling of both mobile and bulk water may be needed to fully understand soil water hydrological processes. Additionally, because of the time scales involved in tree water transport, long term sampling of potential source water may be needed to fully evaluate tree water use via xylem water isotopic composition. Future studies will focus on further exploring the driving forces that could potentially separate tree and soil water.

6. Conclusion

We investigated soil water storage and flow separation in a humid, low energy boreal (Latitude 57^{o}) environment in Scotland. We considered four representative soil-vegetation units that included forested (with Scots Pine (*Pinus sylvestris*)) and non-forested sites, and two widespread soil types (Histosols and Podzols). Firstly, we examined whether subsurface soil water isotopic separation could be identified by comparing stable isotopes of soil water held at different soil water tensions. The isotope analyses showed that water that was held at increasing tensions was slightly more depleted than the more mobile water, and could therefore have been influenced by older (winter) precipitation. These patterns were independent of soil type, vegetation cover, and time during the growing season, although there was a slight tendency towards less separation with soil depth. However, no strong evidence of evaporative fractionation was found, apart from some minor effects in the upper soil profile of the forested Podzol site. Secondly, we explored how water use of vegetation, as identified by the xylem isotope composition, compared to the soil water stable isotopes. Overall, these data showed that xylem water was largely on the meteoric water line and similar to soil and stream water. This suggests that there was no marked separation between these pools and no evidence to support the 'two water worlds' hypothesis. However, for the two soil types we found contrasting results which, for the Podzol implied uptake of evaporated water from the near surface (upper 10cm) soil horizons where fine root densities are concentrated. The trees of the Histosol site seemed to further exhibit a delayed response in their isotopic composition of xylem water compared to the soil water, possibly suggesting low rates of water uptake.

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Table 1. Hydroclimatological and soil moisture conditions during the extensive soil and vegetation water sampling campaigns during the growing season

Hydroclimatological Conditions							
	Units	**11 June 2013**			**22 July 2013**		
Mean Air Temperature	°C	9.76			14.94		
Potential Evapotranspiration	mm d^{-1}	1.86			3.19		
Discharge	mm d^{-1}	0.79			0.41		
Precipitation	mm d^{-1}	0.32			0		
Precipitation in Previous 3 days	mm	0			0		
Precipitation in Previous 7 days	mm	0			0		
Precipitation in Previous 14 days	mm	9.53			0		
Volumetric Soil Moisture Conditions (Daily Mean)							
	Units	**11 June 2013**			**22 July 2013**		
Depth (m)		**-0.1**	**-0.3**	**-0.5**	**-0.1**	**-0.3**	**-0.5**
Hs (Histosol/Sphagnum)	-	0.81			0.78		
Hf (Histosol/Scots Pine)	-	0.54			0.47		
Ph (Podzol/Heather)	-	0.31	0.26	0.25	0.23	0.22	0.21
Pf (Podzol/Scots Pine)	-	0.36	0.32	0.22	0.16	0.18	0.15

Table 2 Observed deuterium ranges in vegetation and soil water for all sites durig the two extensive sampling campaigns during the growing season. Range values show min, max (mean). June and July samples are bulked.

Sampling		Hs		Hf		Ph		Pf	
		n	range	n	range	n	range	n	range
Vegetation		0	NA	6	-62.4,-48.8 **(-57.3)**	0	NA	5	-62.9, -41.8 **(-53.6)**
0.1 m Depth	Mobile	4	-49.8,-45.1 (-47.4)	4	-53.8,-46.4 (-50.8)	3	-43.7,-42.8 (-43.3)	3	-48.8,-45.1 (-46.7)
	< 200kPa	5	-58.3,-54.5 (-56.3)	5	-56.9,-54.0 (-55.6)	0	NA	0	NA
	< 700kPa	6	-57.7,-55.1 (-56.3)	7	-56.7,-53.9 (-55.2)	2	-44.3,-44.0 (-44.1)	1	-38.6
	< 1100kPa	7	-57.6,-54.2 (-56.5)	7	-57.2,-53.3 (-55.2)	3	-45.4,-44.2 (-44.7)	1	-39.3
	200 - 1100kPa	2	-56.1,-55.7 (-55.9)	2	-56.7,-55.8 (-56.3)	0	NA	0	NA
	Bulk	2	-59.6,-59.4 (-59.5)	2	-60.6,-54.8 (-57.7)	2	-55.1,-53.5 (-54.3)	2	-53.1,-52.5 (-52.8)
0.3 m Depth	Mobile	4	-58.2,-57.8 (-58.1)	4	-56.5,-54.9 (-55.9)	4	-52.8,-46.4 (-49.6)	3	-49.1,-48.7 (-48.9)
	< 200kPa	6	-59.4,-55.9 (-57.6)	5	-56.7,-54.3 (-55.2)	0	NA	0	NA
	< 700kPa	7	-58.0,-55.6 (-56.8)	7	-58.1,-53.1 (-55.9)	2	-46.2,-44.6 (-45.4)	1	-47.7
	< 1100kPa	6	-59.1,-55.5 (57.2)	7	-53.8,-53.3 (-56.3)	2	-45.8,-45.1 (-45.5)	1	-47.0
	200 - 1100kPa	3	-56.4,-55.7 (-56.0)	2	-56.7,-55.8 (-56.3)	0	NA	0	NA
	Bulk	2	-61.8,-59.5 (-60.6)	2	-60.8,-60.7 (-60.7)	2	-55.7,-54.7 (-55.2)	2	-63.6,-60.4 (-62.0)
0.5 m Depth	Mobile	0	NA	4	-57.9,-55.8 (-56.8)	2	-49.1,-48.9 (-49.0)	2	-55.1,-54.5 (-54.8)
	< 200kPa	8	-58.7,-55.9 (-57.4)	7	-58.0,-53.5 (-55.9)	0	NA	0	NA
	< 700kPa	8	-58.7,-56.9 (-57.9)	7	-57.6,-52.5 (-55.8)	2	-47.3,-46.9 (-47.1)	1	-53.4
	< 1100kPa	8	-60.1,-57.0 (-58.4)	8	-56.8,-54.1 (-55.7)	2	-48.2,-46.9 (-47.5)	1	-51.8
	200 - 1100kPa	3	-57.5,-56.0 (-56.6)	1	-56.3	0	NA	0	NA

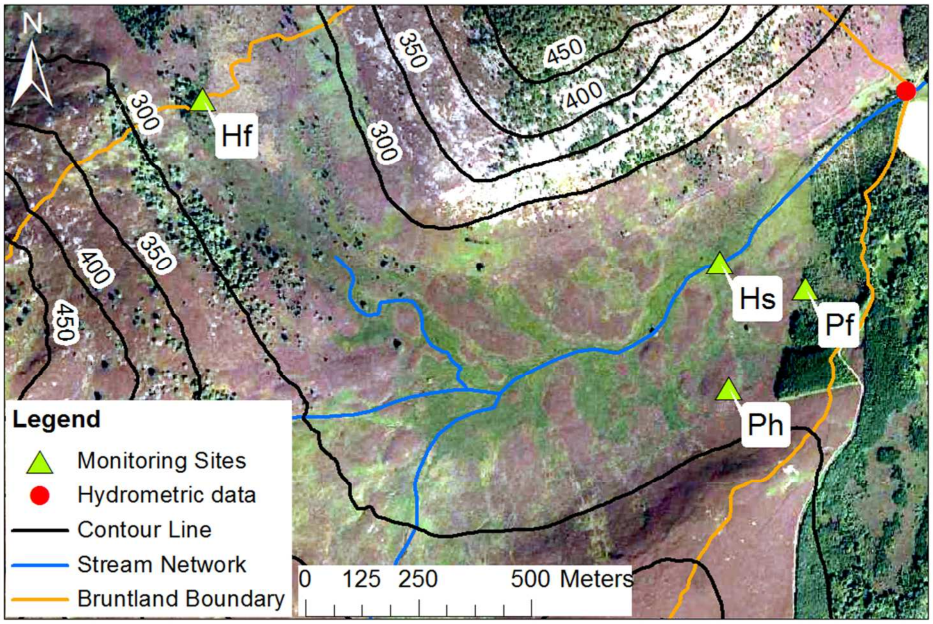

Figure 1 Bruntland Burn study area, showing the four monitoring sites, the topography, and vegetation distribution, where light green areas are peat bog, purple heather vegetation on freely draining soils, and dark green areas are forested.
190x142mm (300 x 300 DPI)

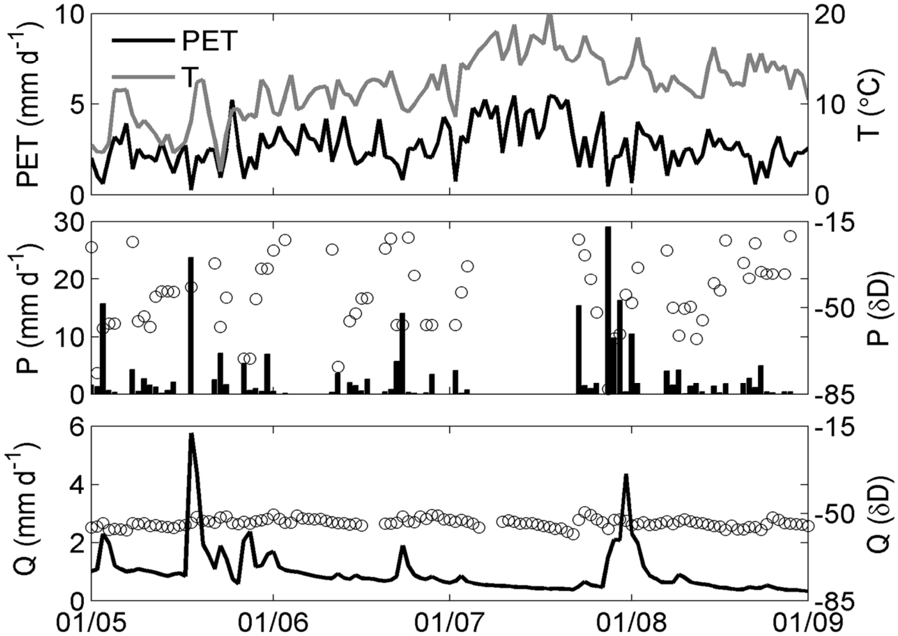

Figure 2 Hydroclimatic data (potential evapotranspiration, temperature, precipitation, and discharge) plots for May-August 2013, and deuterium data for catchment precipitation inputs and discharge outputs.
190x142mm (300 x 300 DPI)

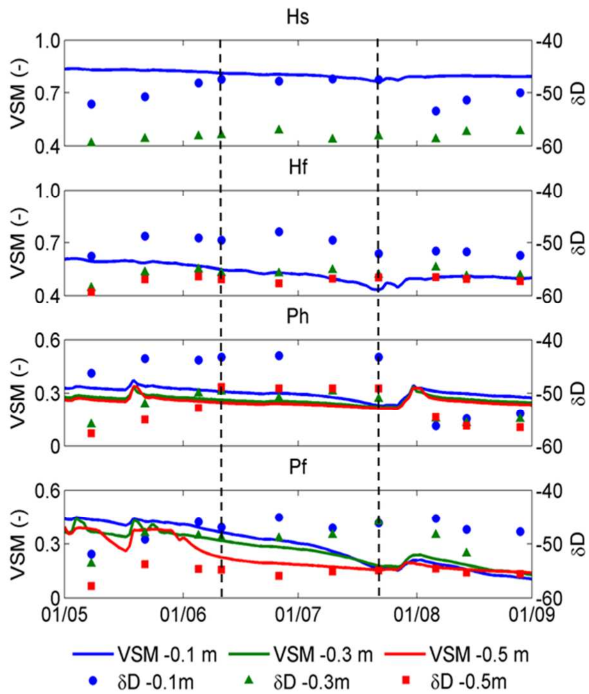

Figure 3. Temporal dynamics in volumetric soil moisture (VSM) and deuterium timeseries for the May - August 2013 period. Data for three sampling soil depths are shown (-0.1 m in blue, -0.3 m in green, and -0.5 m in red) for the four sampling sites: Histosols with Sphagnum (Hs) and Forest (Hf) cover, and Podzols with Heather (Ph) and Forest (Pf) cover. The dotted lines indicate times of sampling for other soil water extraction methods.
190x142mm (300 x 300 DPI)

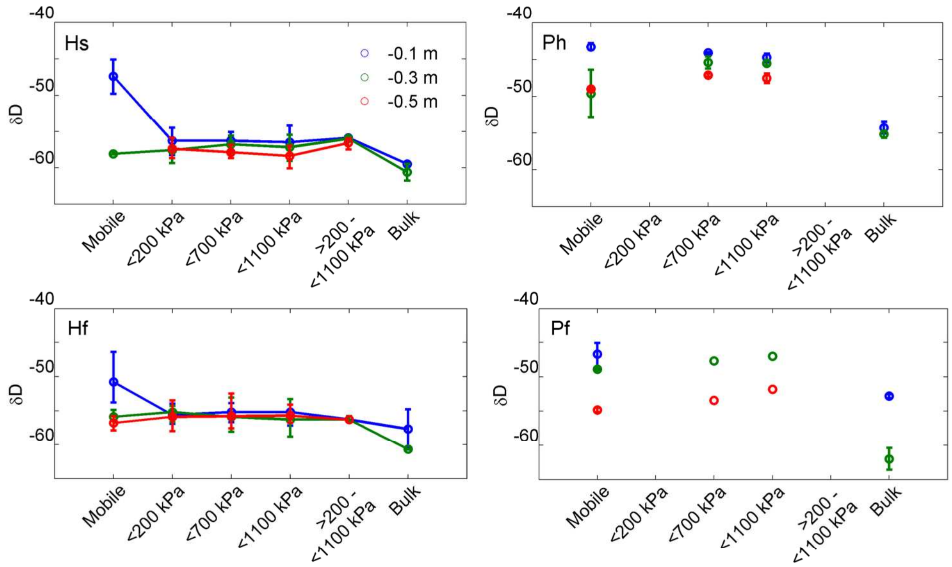

Figure 4. Soil water deuterium signatures for water extracted at different tensions. Data for three sampling soil depths are shown (-0.1 m in blue, -0.3 m in green, and -0.5 m in red) for the four sampling sites: Histosols with Sphagnum (Hs) and Forest (Hf) cover, and Podzols with Heather (Ph) and Forest (Pf) cover. June and July samples are bulked.
190x142mm (300 x 300 DPI)

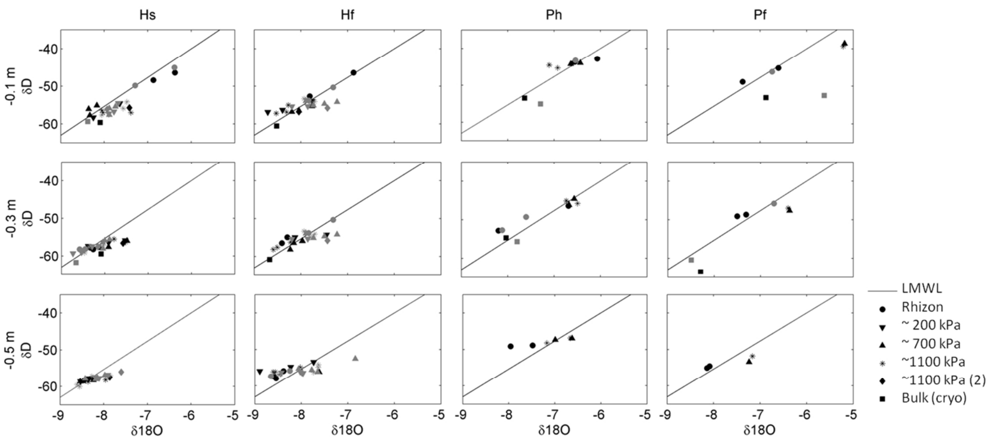

Figure 5. Soil water data for the four monitoring sites and three soil depths using different water extraction techniques representing water held at different soil water tensions. The black data are for the June collection; the grey for the July collection.
190x142mm (300 x 300 DPI)

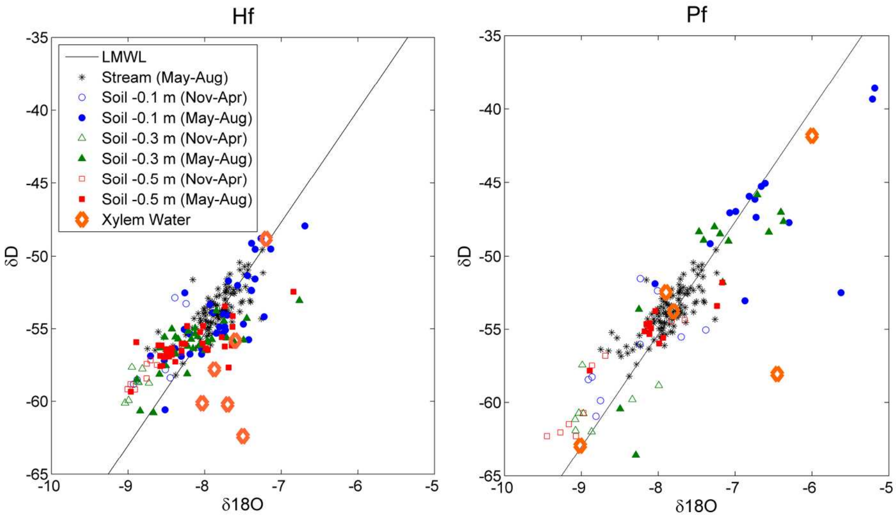

Figure 6. Isotope composition for stream water, soil water at the three sampling depths, and xylem water for tree covered sites with Histosol (Hf) and Podzol (Pf) soil types. The filled soil water symbols include mobile Rhizon, centrifuged and cryogenically extracted samples collected during the 2013 growing season. Open symbols indicate mobile soil water collected during the winter prior to the main monitoring period.
179x100mm (300 x 300 DPI)