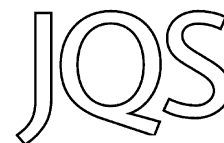


Measurements of hydrogen, oxygen and carbon isotope variability in *Sphagnum* moss along a micro-topographical gradient in a southern Patagonian peatland



N. J. LOADER,^{1*} F. A. STREET-PERROTT,¹ D. MAUQUOY,² T. P. ROLAND,³ S. VAN BELLEN,² T. J. DALEY,⁴ D. DAVIES,¹ P. D. M. HUGHES,⁵ V. O. PANCOTTO,⁶ G. H. F. YOUNG,¹ M. J. AMESBURY,³ D. J. CHARMAN,³ G. MALLON⁷ and Z. C. YU⁸

¹Department of Geography, College of Science, Swansea University, Swansea SA2 8PP, UK

²School of Geosciences, University of Aberdeen, Aberdeen AB24 3UE, UK

³Geography, College of Life and Environmental Sciences, University of Exeter, Exeter EX4 4RJ, UK

⁴School of Geography, Earth and Environmental Sciences, Plymouth University, Plymouth PL4 8AA, UK

⁵Department of Geography, University of Southampton, Southampton SO17 1BJ, UK

⁶CADIC- CONICET, Bernardo Houssay 200, Ushuaia, Tierra del Fuego, Argentina

⁷Department of Geography, University of Sheffield, Sheffield S10 2TN, UK

⁸Department of Earth and Environmental Sciences, Lehigh University, Bethlehem, PA 18015, USA

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ABSTRACT: Peat archives offer a diverse range of physical and chemical proxies from which it is possible to study past environmental and ecological changes. Direct numerical calibration and verification is difficult so process-based and mechanistic studies are therefore required to establish and quantify links between environmental changes and their associated proxy-responses. Traditional ‘space-for-time’ calibrations provide a solution to this calibration problem, but are often unable to isolate a single environmental variable from other potentially confounding variables. In this study, we explored the potential of a site-specific ‘space-for-time’ approach applied to a hummock-hollow transect on an ombrotrophic raised bog in Patagonia, southern Chile. Coupled stable carbon, oxygen and hydrogen isotopic measurements were made on individual samples of *Sphagnum* moss cellulose and compared with plant-associated waters, local hydrology, temperature and relative humidity, sampled at the same points along the study transect. Results reveal a range of environmental responses, which were supported by plant-physiological models in the case of carbon and oxygen isotopes. For hydrogen isotopes, the results obtained from cellulose indicated a need for further research into hydrogen isotope fractionation in *Sphagnum*. We recommend conducting site-specific characterization of plant response to support the development of peat-based isotope records for palaeoenvironmental research, and where logistically possible, that monitoring is conducted over timescales appropriate to the time-integrative nature of the *Sphagnum* record.

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KEYWORDS: methanotrophy; palaeoclimate; Patagonia; *Sphagnum*; peat; South America; stable isotopes.

Introduction

Terrestrial archives of palaeoenvironmental change are proving to be important tools in our understanding of the Earth system, its long-term variability, and likely response to natural and anthropogenic disturbance. Under favourable circumstances, changes in the physical and chemical properties of such archives can be linked unambiguously to environmental controls, and assuming uniformitarianism, it is possible to reconstruct past environmental changes through the duration of the record.

The accumulation of peat in ombrotrophic (rain-fed) mires provides an archive of both physical and chemical information that may be related both qualitatively and quantitatively to environmental variability (Richardson, 1986; Barber *et al.*, 1994; Mitchell *et al.*, 2001; Booth and Jackson, 2003; Mauquoy *et al.*, 2004; Charman *et al.*, 2006, 2009; Daley *et al.*, 2009; Loisel *et al.*, 2009; Moschen *et al.*, 2009; van der Knaap *et al.*, 2011; De Vleeschouwer *et al.*, 2012; Mazier *et al.*, 2012; Loisel and Yu, 2013; Chambers *et al.*, 2014; Swindles *et al.*, 2015). Many significant features of the peat archive provide unrivalled opportunities for palaeoecological study, including a capacity for multi-proxy investigation,

widespread geographical distribution, typically rapid accumulation rates (when compared with many sedimentary archives), minimal bioturbation and excellent potential for age control. Unfortunately, despite the best efforts of Quaternary researchers, the absence of annual resolution (however fine the sampling resolution), inter- and intra-site replication, and unavoidable chronological uncertainties mean that it is virtually impossible to conduct robust calibration and verification against instrumental meteorological data in a manner similar to that used to calibrate annually resolved proxies (Charman *et al.*, 2007; Jones *et al.*, 2009; Barnekow *et al.*, 2010; Amesbury *et al.*, 2011, 2012a,b; Blaauw and Mauquoy, 2012; Swindles *et al.*, 2013). Consequently many peat-based reconstructions display limited quantifiable reconstructive skill (NRC, 2006).

To address such constraints would require significant long-term investment and site-specific monitoring of peat accumulation, *Sphagnum* physiology and hydro-climate over many decades, which is currently unfeasible. A commonly used method for resolving this calibration issue is the ‘space-for-time’ approach, where multiple sites are sampled across an ecological or climatological gradient. This provides a range of plant responses across a very wide range of environmental conditions, as might be expected if a single site were monitored over many decades (e.g. Ménot and

*Correspondence: N. J. Loader, as above.

E-mail: n.j.loader@swansea.ac.uk

Burns, 2001; Ménot-Combes *et al.*, 2002; Loader and Hemming, 2004; Loader and Rundgren, 2006; Skrzypek *et al.*, 2007, 2011; Amesbury *et al.*, 2015).

This approach to calibration provides valuable insights into signal preservation, but also has significant limitations. Primarily, care must be taken to ensure that only a single variable changes across the gradient. In practice, considering the diversity of peat-bog ecology, this is a very difficult condition to satisfy as there is a risk that co-varying environmental factors will obscure or inflate any resulting calibration. Similarly, physical parameters or differences in altitude, air mass trajectory, nutrient input (pollution), UV exposure, hydrology, disturbance and seasonality in plant growth may also vary to differing degrees across large spatial gradients and can *in extremis* yield over-optimistic, atypical or incorrect impressions of climatic control as artefacts of such an approach.

An example of this phenomenon may be identified in early isotope dendroclimatology, where for practical reasons, spatial calibrations were first employed to explore the potential of the proxy. High correlations were obtained between tree-ring chemistry and both meteorological and isotopic data over large geographical ranges (e.g. $r=0.97$, Yapp and Epstein, 1982; $r=0.88$, Burk and Stuiver, 1981). Although these early studies were informative, the environmental gradients over which they were conducted were so large that they masked the natural intra-site and inter-tree variability, presenting a very optimistic picture of signal strength (McCarroll and Loader, 2004; Loader *et al.*, 2013a,b). The same is likely to be true for non-annually resolved sedimentary archives calibrated in this way. The potential of peat as a multi-proxy archive of past environmental change remains significant, but improvements to the characterization and quantification of the environmental signals represented and their natural variability within and between sites are required and should remain a research priority (Loisel *et al.*, 2009).

For oxygen and hydrogen isotopes, the process models proposed by Roden *et al.* (2000) and Saurer *et al.* (2002) remain valid for *Sphagnum*, but without isotopic modification of plant water through changes in stomatal aperture. For an ombrotrophic bog in northern Newfoundland, Daley *et al.* (2010) demonstrated a close association between the oxygen isotopic composition of *Sphagnum* cellulose and the oxygen isotopic composition of local precipitation, suggesting that where air humidity is near 100% the isotopic composition of cellulose provides a direct proxy for meteoric water. However, Price *et al.* (2009) examined isotopic gradients and diffusion of moisture through peat profiles and concluded that the relationship is somewhat more complex. Through a series of laboratory experiments, they were able to demonstrate evaporative enrichment of water vapour in the upper c. 5 cm of their simulated acrotelm, which may indicate that the water isotopes record not only the isotopic composition of recent meteoric waters, but also an evaporative/recharge component from depth.

Modifications to *Sphagnum* oxygen and hydrogen isotopic compositions may therefore result from local changes in relative humidity, temperature, light and water-table depth (hydrology) as well as from changes in the isotopic composition of precipitation (source) water. If these controls can be quantified, the isotopic composition of *Sphagnum* from palaeo-records could be used to reconstruct the environmental history of individual mires (semi-)quantitatively. The resulting records of environmental change could then be employed to evaluate isotope-enabled climate models and to test inferences drawn from large-scale transfer functions or from ecological indicators such as humification, testate amoebae and plant macrofossils, via a multi-proxy approach.

Sphagnum mosses follow the C3 photosynthetic pathway. Hence, the process of carbon isotopic fractionation may be described using the model proposed by Farquhar *et al.* (1982). The carbon/isotope ratio of the photosynthate (and ultimately cellulose) is determined by the isotopic composition of the source carbon dioxide (CO₂), the net conductance of CO₂ to the site of photosynthesis and biochemical fractionation during biosynthesis. Unlike vascular plants, however, *Sphagnum* mosses are unable to regulate gas exchange and moisture loss as they lack the guard cells required to reduce stomatal aperture size during times of moisture stress (Farquhar *et al.*, 1982; Proctor *et al.*, 1992; Rice and Giles, 1996; Rice, 2000; Ménot and Burns, 2001; Price *et al.*, 1997). Instead, during photosynthesis, carbon dioxide freely enters the plant through pores but must then diffuse through a surface water-film on its way to the chloroplast where photosynthesis occurs (Loisel *et al.*, 2009). The isotopic effect of this diffusion is to fractionate the CO₂. Under 'wet' conditions, when the thickness of the water film is greatest, decreased fractionation in favour of the lighter isotopes results in isotopically less ¹³C-depleted photosynthates (and cellulose). During 'dry' conditions, when film thickness is reduced, the opposite is true and more ¹³C-depleted (lower) carbon isotopic values result (Rice, 2000). Simply stated, the *Sphagnum* carbon isotopic response to moisture stress is essentially opposite to that which would be expected in vascular plants that have true stomata.

A further consideration in the analysis of carbon isotopes in cellulose from waterlogged environments is the source of CO₂ used by the plants. Several studies have proposed that a significant proportion of the carbon fixed by the plants was emitted from the bog as methane and subsequently converted to CO₂ through the activity of methanotrophic bacteria. Such methane carbon-source models (Basiliko *et al.*, 2004; Raghoebarsing *et al.*, 2005; Larmola *et al.*, 2010) would predict sustained periods of waterlogging with increased methane production and more $\delta^{13}\text{C}$ -enriched CO₂ because of methanotrophy. Under such circumstances, and in contrast to the atmospheric carbon-source model, $\delta^{13}\text{C}$ would correlate positively with fluctuations in water-table depth. It is therefore possible to test these conflicting hypotheses through this investigation.

In recognition of these potential benefits and the problems associated with large-scale space-for-time calibration described above, this study investigated the relationship between stable isotopes in *Sphagnum* mosses and their environment on an ombrotrophic peat bog in southern Patagonia, Chile, South America. The nature of carbon, oxygen and hydrogen isotopic variability in modern *Sphagnum* was explored across a hummock-pool-hummock transect, and the resulting data were compared against micrometeorological, hydrological and stable isotopic data. As a variant on space-for-time calibration, this approach also has limitations, but by sampling across an environmental gradient proximal to any proposed locus of core retrieval, additional site-specific insights into *Sphagnum* isotope variability may be obtained. This can aid interpretation of a specific peat record, as the potentially confounding effects of inter-site ecological and climatological differences reduce significantly because all samples on the transect will have experienced similar precipitation, nutrient status and climate.

Methods

The hummock-hollow-hummock transect was located on a raised *Sphagnum magellanicum* bog complex in the Laguna Parrillar National Reserve (53°23'47.6"S, 71°14'55.2"W,

~309 m elevation) (Fig. 1). Pool microforms were dominated by *Sphagnum falcatulum* with occasional *Carex curta*. *Tetroncium magellanicum* fringes pools, whereas *Sphagnum magellanicum* is dominant and *Marsippospermum grandiflorum* is frequently present on the crests of hummocks. Hummock tops also contained abundant *Empetrum rubrum* with *Gaultheria pumila* and occasional *Nothofagus antarctica*. Several studies have identified both the presence and the absence of inter-specific differences in *Sphagnum* isotopic response (Rice, 2000; Ménot and Burns, 2001; Ménot-Combes *et al.*, 2002; Loisel *et al.*, 2009; Brader *et al.*, 2010; Daley *et al.*, 2010), so to avoid potential species-related differences only *Sphagnum magellanicum* capitula were sampled in this study.

A typical hummock–hollow system with a 66-cm vertical elevation difference was established across a 9.86-m-long N–NNW (008°–341°) orientated transect (Fig. 1). Temperature and humidity loggers (iButton™, Maxim Integrated, Dallas, TX, USA) were positioned along the transect at 1 cm above the moss surface and at ~0.8-m intervals. A total of 16 loggers was deployed along the transect (Fig. 1). The loggers were set to record at 15-min intervals for a period of 5 days

(from 16:00 h on 10 January to 13:00 h on 16 January 2015). At the end of the monitoring period the depth from the moss surface to the water-table was determined. The deepest water-table depth (hummock) was 61 cm below the moss surface (iButton5), and the shallowest (7 cm) was recorded for a low lawn microform fringing a pool (iButton9). The iButton loggers provide a cost-effective method to record changes in temperature and relative humidity. Although easily programmed and deployed, a study by Sullivan and Booth (2011) linking testate amoebae and local microclimate using iButtons identified and considered some of the current shortcomings and technical limitations of this technology.

To overcome any device-specific bias, all iButtons were exposed to a period of common temperature logged in Chile after the field study and an adjustment made relative to the mean logger reading during the 72-h post-monitoring control period. Where the loggers misread, become saturated or condensation occurs over the humidity sensor, they may read $\geq 100\%$. In such cases, relative humidity data are capped at 100% and cannot be deemed to be as reliable as those for temperature. To minimize this effect, the loggers were located with the sensing surface facing towards the

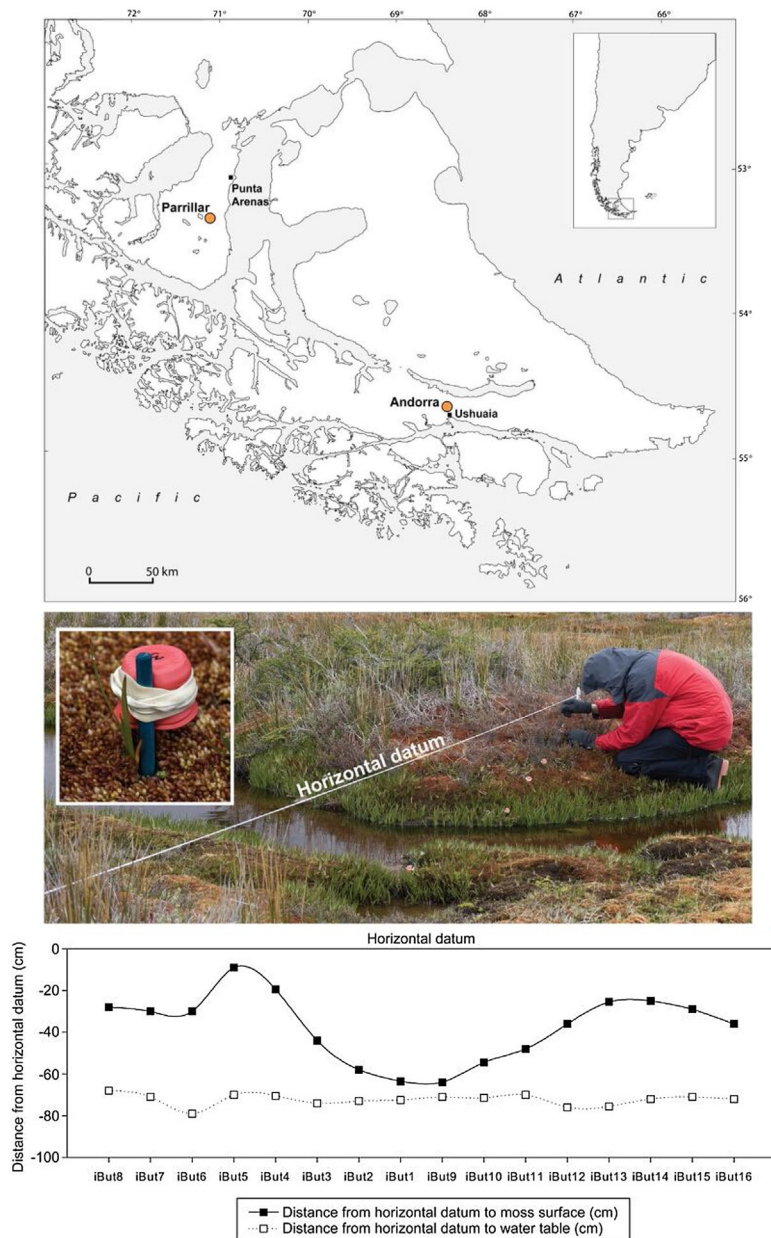


Figure 1. Location of the study site (Parrillar), Andorra Bog and Ushuaia GNP Station (top panel). Photograph of the transect in preparation (middle panel) with shielded iButton™ (inset). Diagram of the profile in cross-section showing relative position of iButton loggers along the transect, microtopography relative to the horizontal datum and depth to water-table as measured in the field (lower panel). The transect was 9.86 m long and orientated N–NNW (008°–341°).

moss and shielded by a rubber cap to reduce the effects of wind and direct insolation heating. Manufacturer's specifications quote measurement accuracy of $\pm 0.5\%$ for relative humidity and $\pm 0.5^\circ\text{C}$ for temperature (Maxim Integrated). During the 72-h control phase at the end of the experiment, the mean precision ($\pm\sigma_{n-1}$) of the 15 working loggers for temperature and relative humidity was $\pm 0.1^\circ\text{C}$ and $\pm 0.7\%$, respectively.

At the end of the monitoring phase, samples of *Sphagnum* moss capitula were collected around each logging point. At least 10 plants were picked for isotopic analysis. Immediately after picking, samples ($>1\text{ mL}$) were squeezed using a syringe to remove internal leaf-water for isotopic analysis. This approach to retrieving leaf-water is sub-optimal, as surface moisture may be included in the sample and deeply held waters are not removed, but because vacuum distillation was not possible on-site, physical removal of this water (hereafter termed plant-associated water) was deemed to provide an indication of relative isotopic changes across the gradient; these changes may be closely related to leaf-water *sensu stricto* and subsequently linked to the samples of precipitation collected at the bog and to the isotopic composition of the *Sphagnum* cellulose. The remoteness of the Parrillar site precluded a longer-term collection of samples to characterize intra-annual isotopic trends. To complement this dataset, monthly samples of summer precipitation and plant-associated water were collected (IAEA, 2014) at a second location in Argentina (Andorra Bog, $54^\circ 37'\text{S}$, $67^\circ 46'\text{W}$, $\sim 340\text{ m}$ elevation) (Fig. 1) approximately 220 km from the study site. This second location experiences a different climate from Parrillar, but enables a comparison to be made of the isotopic variability in plant-associated waters from this more accessible site with monthly data from the International Atomic Energy Agency Global Network of Isotopes in Precipitation (GNIP) station at Ushuaia ($54^\circ 60'\text{S}$, $68^\circ 160'\text{W}$ $\sim 23\text{ m}$ elevation) which may help to inform the results from Parrillar (Fig. 1).

Cellulose was extracted using an acidified sodium chlorite solution and sodium hydroxide (Green, 1963; Loader *et al.*, 1997), modified for *Sphagnum* by Daley *et al.* (2010). Samples were homogenized using an ultra-sonic probe (Laumer *et al.*, 2009) and vacuum dried before isotopic analysis. Dry cellulose (0.30–0.35 mg) was weighed into silver-foil capsules and analysed simultaneously for non-exchangeable hydrogen, oxygen and carbon stable isotopes ($\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$), by high-temperature pyrolysis over glassy carbon at 1400°C using a recently developed online equilibration system (Loader *et al.*, 2015). The ability to determine $\delta^2\text{H}$, $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ in cellulose simultaneously in a single sample provides direct linkage between carbon- and water-use and isotopic variability in *Sphagnum*, thereby affording significant analytical advantages through simpler chemical pretreatment, reduced sample requirements and increased capacity for replication (Loader *et al.*, 2015). Data are reported as per mille (‰) deviations from the VPDB (carbon) or VSMOW (oxygen and hydrogen) standards, using standard delta (δ) notation (Coplen, 1995). Analytical precision determined for the three isotopes is typically better than ± 0.15 , ± 0.30 and $\pm 3.0\%$ for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively (σ_{n-1} $n=10$). Samples of bog and plant-associated waters were stored in a refrigerator (4°C) before analysis by high-temperature pyrolysis at Lancaster University, UK. Analytical precision for this method is typically $\pm 0.5\%$ for oxygen and $\pm 1\%$ for hydrogen isotopes (D. Hughes, pers. comm.).

To establish the nature of inter-plant isotopic variability, inform interpretation and characterize the error and uncertainty in sampling peat records for stable isotope analysis, an

additional sample of *Sphagnum* capitula was collected from a 20-cm² area of emergent *Sphagnum* plants from a raised peat bog (Cors Coch) near Llanllwch, Carmarthenshire, UK ($51^\circ 50'41.2''\text{N}$, $4^\circ 22'25.5''\text{W}$, 25 m elevation). A total of 102 individual plants were isolated, rinsed in deionized water and the capitula removed using a scalpel. Cellulose was then extracted from each plant capitulum and analysed as described above. The variability of the resulting isotopic data was then explored using a replicate resampling (bootstrap) method of 1000 iterations to provide a measure of 'typical' inter-plant variability (Quenouille, 1949; Cauty and Ripley, 2009) (Supplementary Information, Table S1).

Results and discussion

Inter-plant isotopic variability

Stable isotopic measurements of the 102 UK plant capitula revealed a range in isotope variability of 1.7, 2.3 and 2.0‰ for $\delta^{13}\text{C}$, $\delta^{18}\text{O}$ and $\delta^2\text{H}$, respectively (and hereafter). The bootstrap resampling method demonstrated that the variability in plant isotopic data followed a near-normal distribution and showed that when >10 plants are sampled (as in this study) the uncertainty around the resulting mean (1σ) value approached that of the analytical precision of the method (Samples σ_{n-1} : ± 0.11 , ± 0.16 , $\pm 2.77\%$; Method σ_{n-1} : ± 0.15 , ± 0.30 , $\pm 3.0\%$, respectively). When the number of plants incorporated into a single sample exceeded 20 then the sample mean approached the population mean with improved precision (± 0.08 , ± 0.11 , $\pm 2.03\%$) (Table S1). These results are similar to those reported previously for oxygen and carbon isotopes in tree ring cellulose (Loader *et al.*, 2013b). Where individual plant stems are picked and pooled for isotopic analysis, the observed inter-plant isotopic variability reported here may be useful in determining and assigning a measure of plant-associated uncertainty for resulting isotopic time series in cases where the number of plants sampled is low (<20).

Micro-meteorology

Figure 2 shows the diurnal variability in the temperature and relative humidity profiles along the Parrillar transect over the 5-day monitoring period. Of the 16 loggers deployed, one (position 10) failed to record data and so for the purposes of this study, when making comparisons between isotope data and microclimate, data for position 10 are interpolated as the arithmetic mean of the results between loggers 9 and 11. Clear differences in both temperature and relative humidity were logged across the profile, which probably reflected differences in exposure and aspect. Differences between loggers were larger during the daytime (12 h $\sigma_{n-1} \pm 1.8^\circ\text{C}$, $\pm 6.1\%$, $n=15$) than at night (0 h $\sigma_{n-1} \pm 0.6^\circ\text{C}$, $\pm 2.9\%$, $n=15$); in general, the daily amplitude was greater for the hummocks than for the hollows, which may reflect their more exposed topography, greater aerodynamic roughness and capacity to provide shade/shelter.

The mean daily temperature and relative humidity for all working loggers during the monitoring period (from 16 h on 10 January to 13 h on 16 January 2015) were 10.8°C and 85.4%, with inter-logger standard deviations of 0.8°C and 3.6%, respectively. Full details are provided in Table S2.

Water table depth

The depth to the water-table was measured along the transect and found to vary from 7 cm (iButton logger 9) to 61 cm below the moss surface (iButton logger 5). A greater distance to the saturated zone implies a greater moisture deficit and

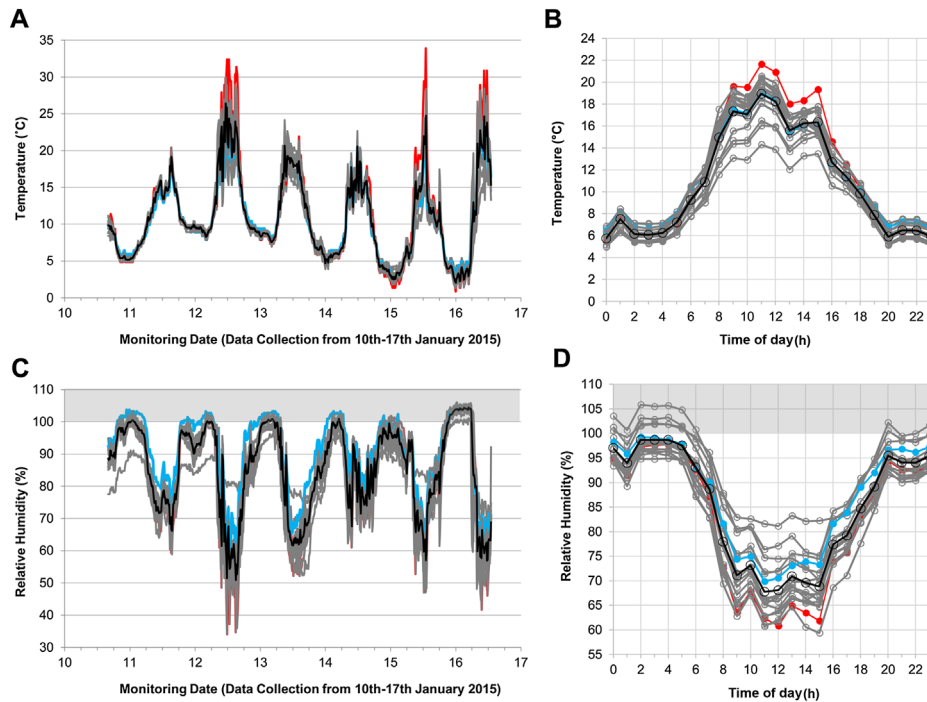


Figure 2. Temperature (A,B) and relative humidity (C,D) datasets at individual iButton™ locations. Data are recorded at every 15 minutes for a period of 5 days from 16:00h on 10 January 2015 to 13:00h on 16 January 2015. B and D show diurnal cycles at hourly averages for the monitoring period for each iButton. The black line shows the mean value measured along the transect. The red line shows data from iButton 5 positioned on the hummock location with the greatest depth to the water-table (61 cm below the moss surface). The blue line shows data from iButton 9 positioned on a low lawn microform fringing a pool; the location with the least depth to water-table (7 cm from the moss surface). Relative humidity data >100% are presented in the grey shaded area to demonstrate the nature of the dataset retrieved and possible systematic over-read of the humidity loggers. iButton data are presented in Table S2.

more evaporative enrichment of water isotopes in the moss plants (Price *et al.*, 2009). Variations in water-table depth during the last 2000 years reconstructed using testate amoebae from three sites in this region typically range from 0 to 70 cm, so this study covered a realistic range of micro-environments (Van Bellen *et al.*, 2014, 2016). Water table has the capacity to fluctuate considerably throughout the year; we recognize the limitation of our single measurement of this variable. While this micro-transect approach does permit a direct link to the short-term monitoring conducted, it would be advisable to extend the frequency and duration of monitoring to attain a more complete picture of environmental controls on *Sphagnum* stable-isotope variability for all parameters investigated.

Water isotopes

Monthly precipitation, pool water and plant-associated waters from Andorra Bog (sampled between May 2012 and January 2014) located 220 km south-east of Parrillar, demonstrate a precipitation-isotope composition similar in seasonal pattern to that recorded at the International Atomic Energy Agency Global Network of Isotopes in Precipitation (GNIP) station at Ushuaia (ca. 10 km south-west of Andorra). GNIP data from Ushuaia are unavailable for the period of this study, but the slope of the regression line between the combined oxygen and hydrogen isotopes (the local meteoric water line: LMWL) at Andorra agrees with the longer-term GNIP precipitation record (Daley *et al.*, 2012). Precipitation and waters collected from *Sphagnum* capitula at Andorra (gradient: 6.27 and 6.03, respectively) plot close to the GNIP data (summer and annual gradients: 6.4 and 6.7, respectively), suggesting that the capitula waters were only slightly enriched or modified during sampling. The pool waters plot within the same data cluster, but with a slightly lower gradient (5.6), which may indicate increased evaporation from the more exposed open-water surfaces; however, the number and range of observations are likely to be too small to plot a robust trendline (Fig. 3).

The plant-associated waters collected along the Parrillar transect exhibit a lower slope (gradient: 3.0) than the annual

or summer-time LMWL for Ushuaia (Daley *et al.*, 2012) or Andorra, implying that the Parrillar plant-associated waters have undergone more modification/evaporative enrichment due to differences in local environmental factors between Andorra and Parrillar. Interestingly, a more proximal GNIP station at Punta Arenas (53°06'18"S, 70°33'36"W, ~340 m elevation) also exhibits lower annual and summer-time LMWL slopes of 5.7 and 5.3, respectively, which may help to explain the plant-associated water relationships observed in this study. The Parrillar moss samples were collected across a topographic gradient specifically to represent a range of evaporative enrichment. Equally, the single collection of plant-associated water may be highly sensitive to rapid changes in microclimate, and less representative compared to the larger-scale characterization of bog hydrology by monthly average values, which undoubtedly integrates and smooths these short-term signals over time and spatially across the mire.

Regional differences in topography, atmospheric circulation (air mass trajectory) and local differences in micro-topography, micro-climate and the extent to which pool-water represents recharge from the catotelm will affect the isotopic composition of bog waters over longer timescales and will be imprinted on the isotopic composition of the *Sphagnum*. This regional- and local-scale variability is not so problematic that it negates the application of stable isotopes in peat-based palaeoclimate research, but it does suggest that before commencing such a study, a coupled isotopic investigation of local precipitation, plant water and hydrology conducted to characterize isotopic variability across the site could aid interpretation of the peat-isotope record.

Plant cellulose isotopes and environmental variability

Sphagnum $\delta^{18}\text{O}$ values ranged from 19.65 to 24.90‰ along the Parrillar transect (mean value 22.48‰). Mean standard deviation reported for the triplicate measures from each of the 16 sampling locations was 0.27‰. Oxygen isotopes correlated moderately well with measures of temperature and relative humidity (Pearson's r). Strongest correlations

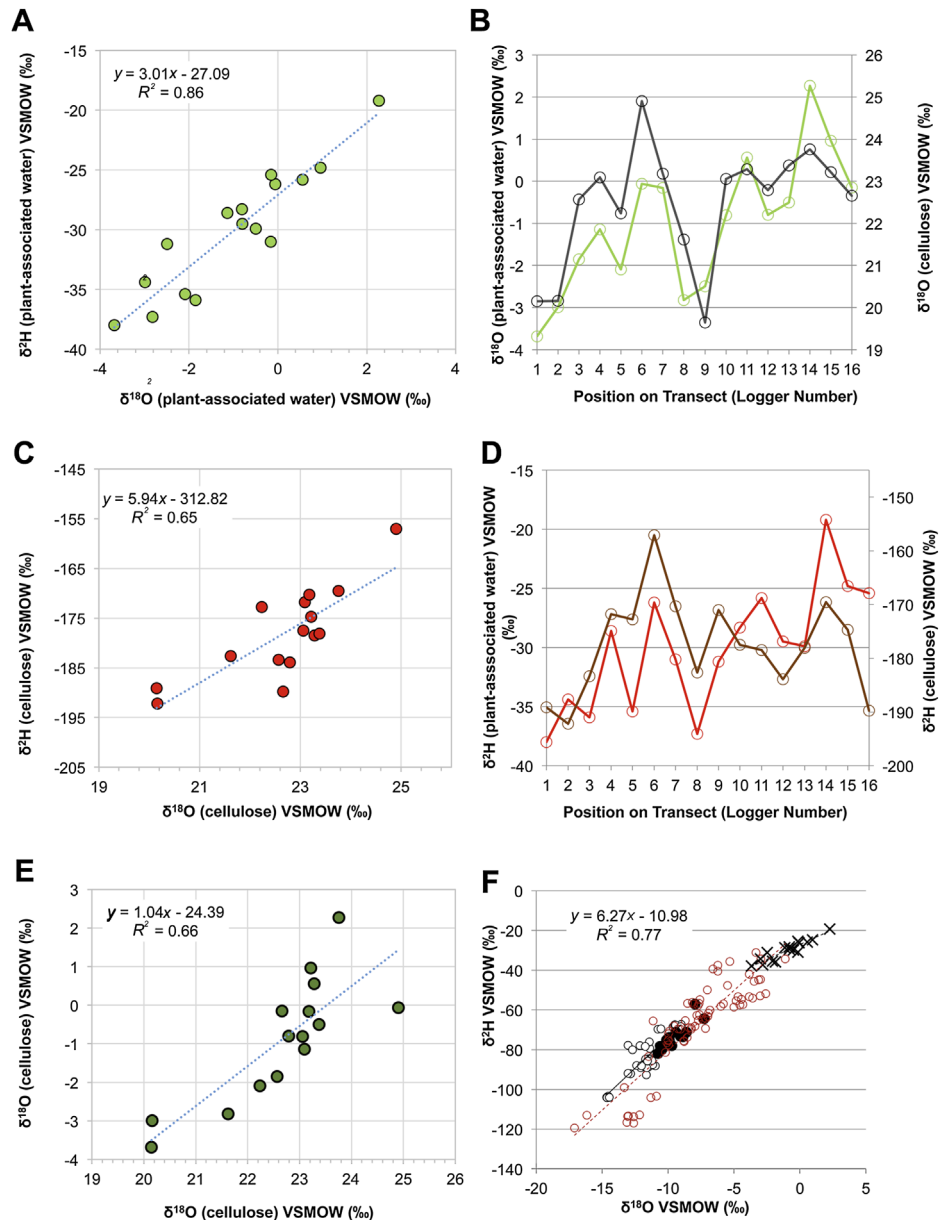


Figure 3. (A) Biplot showing the relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of plant-associated water. (B) The relationship between $\delta^{18}\text{O}$ of cellulose and $\delta^{18}\text{O}$ of plant-associated water plotted as position along the transect. (C) Biplot showing the relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of cellulose. (D) The relationship between $\delta^2\text{H}$ of cellulose and $\delta^2\text{H}$ of plant-associated water plotted as position along the transect. (E) Biplot showing the relationship between $\delta^{18}\text{O}$ of cellulose and $\delta^{18}\text{O}$ of plant-associated water. (F) Biplot showing the relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of meteoric water at Andorra Bog (black open circles), plant-associated water (open red circles) and pool water (filled black circles). Solid trendline and equation show the relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of meteoric water at Andorra for the monitoring period. Dashed trendline shows the relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of plant-associated water. Crosses show the relationship between $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of plant-associated waters sampled along the Parrillar transect.

($r=0.51$ – 0.56 and -0.41 to -0.49 , for temperature and relative humidity, respectively) were obtained during the morning (08:00–12:00 h). A significant correlation ($r=0.80$, $P\leq 0.01$) was observed between the plant-associated water and moss-cellulose $\delta^{18}\text{O}$ values, indicating that the pattern of isotopic variability (or the factors affecting enrichment during the growing season) remained relatively stable influences on the resulting $\delta^{18}\text{O}$ composition of the *Sphagnum* (Fig. 4A,B; Table S3). The isotopic relationship between plant-associated waters and cellulose observed at Parrillar ($\delta^{18}\text{O}_{\text{cellulose}} 0.714\text{‰}$, $\delta^{18}\text{O}_{\text{plant-associated water}} +23.18\text{‰}$) shows that despite the strong correlation between plant-associated water and cellulose oxygen isotope composition, the plant-associated water sampled in this study was modified/evaporatively enriched relative to the net isotopic composition of the water used to form the cellulose (Fig. 3). The simple model of *Sphagnum* cellulose reflecting a source (plant-associated water) enriched by 27‰ (DeNiro and Epstein, 1981) is not supported. This is probably in part an artefact of the sampling procedure for plant-associated waters, but is likely also to indicate complexity in the climate–*Sphagnum* relationship beyond the ‘aquatic plant’ model (Daley *et al.*, 2010), which requires further detailed investigation and modelling (Saurer

et al., 2002; Treydte *et al.*, 2014). Future studies should explore these differences to refine mechanistic understanding, but nevertheless the strong parallelism between source water $\delta^{18}\text{O}$ and precipitation and the resulting plant cellulose is encouraging. In the absence of numerical calibration in the palaeorecord, the strength of the above correlation is such that the relationship can be scaled to that of the source water without significant loss in signal (McCarroll *et al.*, 2015); in this manner a reconstruction of past variability in plant-associated water might be attainable were this record to be extended back in time. Daley *et al.* (2012) reported only weak associations between $\delta^{18}\text{O}$ in precipitation and temperature for GNIP data for Punta Arenas and Ushuaia. In this study, cellulose $\delta^{18}\text{O}$ values correlated moderately well with water-table depth ($r=0.68$, $P\leq 0.01$), temperature ($r=0.56$, $P\leq 0.05$) and relative humidity ($r=-0.49$, ns $p>0.05$), so it is possible that cellulose $\delta^{18}\text{O}$ was influenced both by large-scale changes in atmospheric circulation and by local site microclimate (Fig. 4A,B).

Cellulose $\delta^2\text{H}$ was more complex to interpret, indicating that elements of hydrogen isotope physiology may require further investigation. *Sphagnum* hydrogen isotopic values range from -157 to -192‰ along the transect (mean

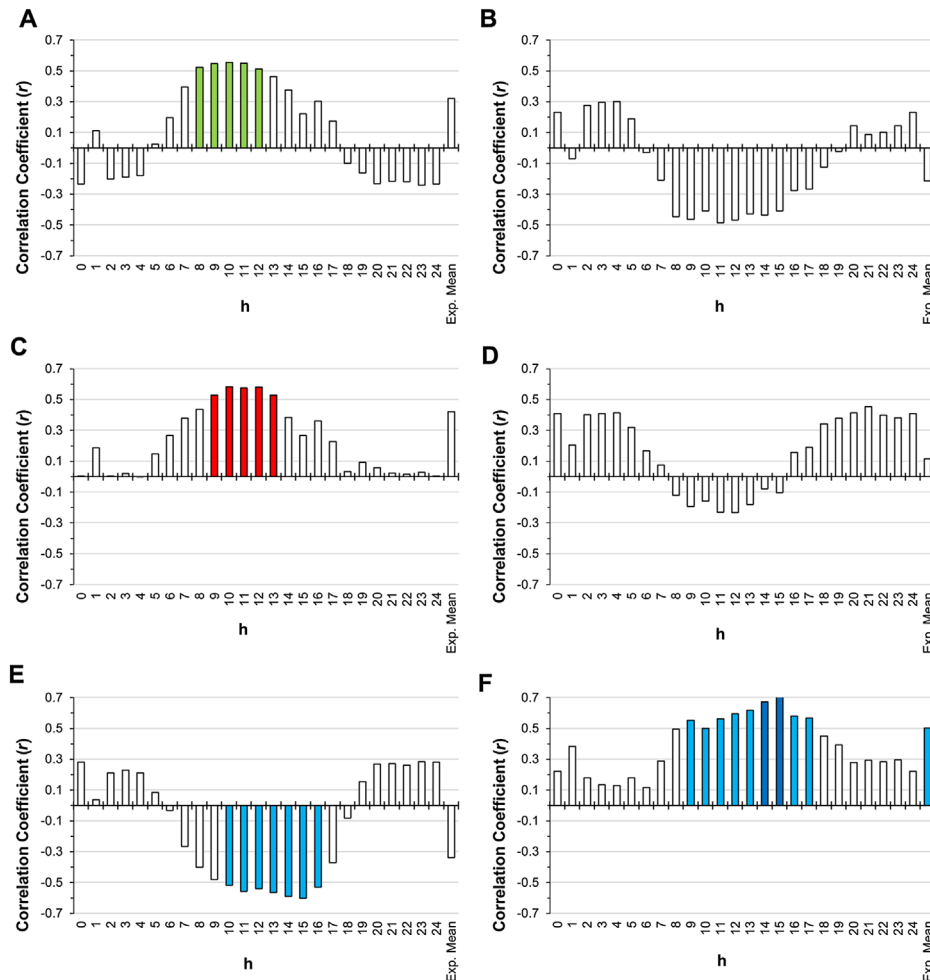


Figure 4. Composite correlation diagrams presenting the Pearson's correlation coefficient for temperature (A, C, E) and relative humidity (B, D, F). Open bars are statistically significant at $P \geq 0.05$ ($n=16$), light shading shows significant correlation at $P \leq 0.05$ ($r \geq 0.497$), dark shading significant at $P \leq 0.01$ ($r \geq 0.623$). (A,B) Oxygen isotopes (*Sphagnum*), (C,D) hydrogen isotopes (*Sphagnum*), (E,F) carbon isotopes (*Sphagnum*). For water-table depth and deuterium excess correlations see Supporting Information.

–178‰). Mean standard deviation reported for the triplicate measures from each of the 16 sampling locations was 1.2‰. Plant-associated water $\delta^2\text{H}$ values correlated well with their paired $\delta^{18}\text{O}$ values ($r=0.93$, $P \leq 0.01$). The gradient of the regression line (3.0) was much lower than that of the local summer LMWL for Ushuaia (6.7) or Punta Arenas (5.3), which might indicate mixing or integration of surface/precipitation waters with deeper bog-water which may have precipitated under a different atmospheric circulation or evaporative regime. Hydrogen isotopes in the leaf-associated waters correlated with daytime (10:00 h) temperature ($r=0.58$, $P \leq 0.05$) but not with relative humidity (Fig. 4C,D). In contrast to oxygen isotopes, the strongest correlations with relative humidity ($r=0.45$, ns , $p > 0.05$) were observed during the night (21:00 h). This interesting result may indicate that plant respiration exhibits a greater influence on $\delta^2\text{H}$ than on $\delta^{18}\text{O}$ and, if so, provides an explanation as to why oxygen and hydrogen isotopes in moss cellulose did not behave as predicted by plant physiological models (Roden *et al.*, 2000; Roden and Ehleringer, 2000). Calculation of deuterium excess (d) for the plant-associated water samples and their comparison with water-table depth provides a measure of evaporation/recycling along the transect. Higher (less negative) d values were observed at logging positions closest to the water-table suggesting that evaporative enrichment increases with depth to water-table ($r=-0.52$, $P \leq 0.05$). This would indicate that differences in bog microtopography and/or microclimate rather than physiology might be responsible for the variability in $\delta^2\text{H}$ observed and its relationship to the LMWL (Fig. S1).

Sphagnum carbon isotope values ranged from –24.6 to –27.5‰ along the Parrillar transect (mean –26.6‰). Mean

standard deviation reported for the triplicate measures from each of the 16 sampling locations was 0.12‰. Physiological models for carbon isotopic fractionation in mosses (Farquhar *et al.*, 1982; Proctor *et al.*, 1992; Rice and Giles, 1996; Rice, 2000; Ménot and Burns, 2001; Price *et al.*, 1997) predict a positive relationship between the moisture status of the plant and its carbon isotope values due to changes in the ease with which CO_2 diffuses to the site of photosynthesis. This pattern is clearly identified in our micro-transect study, in which statistically significant associations with water-table depth ($r=-0.77$, $P \leq 0.01$) relative humidity ($r=0.72$, $P \leq 0.01$) and temperature, ($r=-0.60$, $P \leq 0.05$) (which relate indirectly to moisture stress) were identified. Composite correlation diagrams identify a stronger correlations during daytime, with the period 11:00–15:00 h reporting highest values (Figs 4E,F and 5). This coincides with the period of most intense heat, sunshine (photosynthetically active radiation) and evaporation from the moss surface (lower relative humidity) when the relative differences across the transect are most pronounced and contrasts with lower correlation coefficients during the night.

This result agrees with plant physiological models and the findings of Loisel *et al.* (2009), but runs counter to methane carbon-source models (Basiliko *et al.*, 2004; Raghoebarsing *et al.*, 2005; Larmola *et al.*, 2010) and some larger-scale (non-*Sphagnum*) space-for-time transects where it is possible that uncharacterized factors or inter-site differences may have contributed to the nature of the relationships observed (Ménot and Burns, 2001; Loader and Rundgren, 2006; Skrzypek *et al.*, 2007; Amesbury *et al.*, 2015).

Our results indicated that *Sphagnum* $\delta^{13}\text{C}$ provides useful insights into past changes in bog hydrology, particularly in

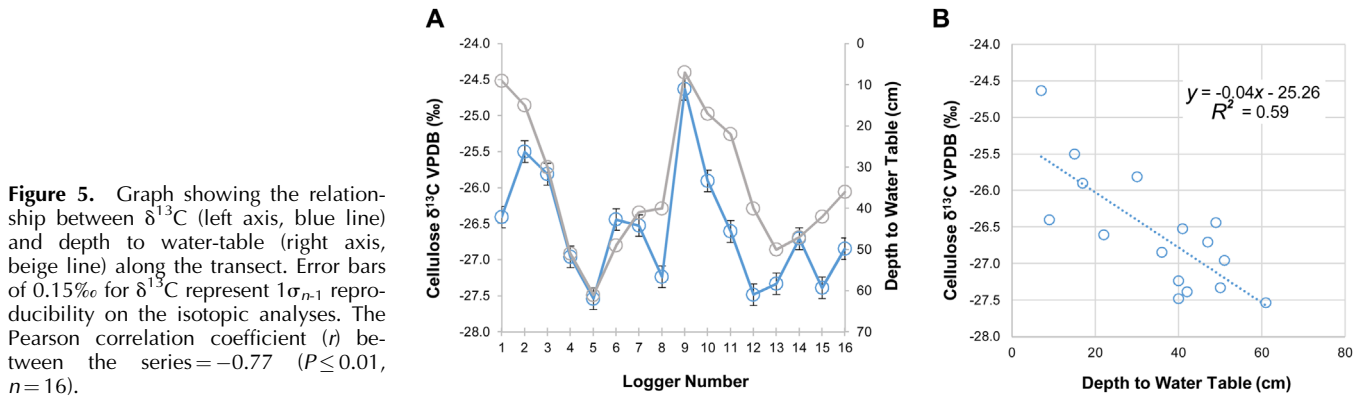


Figure 5. Graph showing the relationship between $\delta^{13}\text{C}$ (left axis, blue line) and depth to water-table (right axis, beige line) along the transect. Error bars of 0.15‰ for $\delta^{13}\text{C}$ represent $1\sigma_{n-1}$ reproducibility on the isotopic analyses. The Pearson correlation coefficient (r) between the series is -0.77 ($P \leq 0.01$, $n = 16$).

areas where plant macrofossil or testate amoeba assemblages exhibit low species diversity or register palaeohydrology with large statistical uncertainties. The Parrillar $\delta^{13}\text{C}$ data are encouraging because the carbon isotope/water-table relationship reported explains a greater proportion of variance in water-table depth than that obtained using testate amoeba assemblages alone ($r^2 = 54$ and 16% , for $\delta^{13}\text{C}$ and testate amoebae, respectively). Similarly, large changes in water-table depth have been reconstructed for southern South American peatlands using testate amoebae, but it is unclear whether these indeed record climatic change or have been influenced by increased UV radiation. As a moisture-sensitive proxy, coupled measurement of *Sphagnum* $\delta^{13}\text{C}$ and testate amoebae may be able to explain the extent to which this signal represents climatic change, increased UV radiation or a combination of the two (Robson *et al.*, 2005; van Bellen *et al.*, 2016).

Conclusions

A space-for-time calibration examining the relationship between the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of local water, temperature, relative humidity, water-table depth and stable isotopic variability ($\delta^{18}\text{O}$, $\delta^2\text{H}$, $\delta^{13}\text{C}$) in *Sphagnum* moss cellulose growing over a hummock-hollow transect in southern Patagonia was conducted with the aim of improving process-based understanding and palaeoenvironmental interpretation of the peat isotopic palaeorecord. Our approach differed from temporal correlation using long instrumental records, which can be unreliable in archives exhibiting variable/low temporal resolution and from larger-scale space-for-time calibrations where single environmental signals are difficult to isolate.

This study assumed that the spatial variability and relative differences observed during the period of monitoring were consistent with the longer-term environmental signals preserved along the transect. Significant potential exists for developing this approach, but it could be improved in future by increasing the duration, replication and spatial extent of the monitoring phase, and including longer-term monitoring of a wider range of environmental variables: for example CO_2 and CH_4 fluxes, isotopes in precipitation, water-table depth and *Sphagnum* growth. An additional methodological enhancement would be to incorporate vacuum distillation of leaf water *sensu stricto*, which is preferable to the physical extraction of plant-associated water employed in this investigation.

Oxygen isotopes in cellulose match the pattern of variability in plant-associated waters collected along the transect, although the relationship between *Sphagnum* plant-associated waters observed at Parrillar does not follow the expected trend, in terms of either the slope or the intercept of the

LMWL. These issues notwithstanding, the close relationship between $\delta^{18}\text{O}$ in precipitation, pool waters, plant-associated waters (Andorra) and plant-associated waters and cellulose (Parrillar) offers exciting opportunities for numerical calibration and Earth system modelling (Daley *et al.*, 2011; Saurer *et al.*, 2014; Frank *et al.*, 2015). However, extension to the grand scale (climate or Earth-system modelling) will require site-based understanding of peat bog isotope hydrology rather than simply assuming that $\delta^{18}\text{O}_{\text{cellulose}}$ can be directly translated into $\delta^{18}\text{O}$ growing-season precipitation.

Hydrogen isotopes yielded the weakest correlations with the measured daytime environmental variables over the full duration of the investigation, but interestingly some of the highest correlations with relative humidity during the 20:00–05:00 h (dark) period may indicate a significant respiration component in the $\delta^2\text{H}$ signal. Variability in deuterium excess (d) along the transect revealed a pattern indicative of evaporative enrichment and recycling linked to the relative exposure and depth to water-table of the sampling points.

In this study conducted over a short monitoring period, we have demonstrated close relationships between local microclimate, source water isotopic composition and the isotopic composition of *Sphagnum* cellulose, informed by mechanistic understanding. There is significant scope for the refinement of this approach, but even in its simplest form, the ability to quantify moss isotopic variability over a range of micro-environmental conditions provides a means with which to quantify and assign measures of statistical uncertainty to *Sphagnum* isotopic data. Chronology in peat sediments can never reach true annual resolution, making direct numerical calibration difficult, so process studies or mechanistic approaches capable of taking into account uncertainties in plant response represent a promising direction for future research.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1. Results obtained from the bootstrap analysis of the 102 individual *Sphagnum* moss capitula cellulose samples from Cors Coch, near Llanllwch, UK. Summary statistics for $\delta^{18}\text{O}$, $\delta^{13}\text{C}$ and $\delta^2\text{H}$.

Table S2. Summary iButtonTM temperature and relative humidity data measured along the transect.

Table S3. Raw data hourly averages and Pearson's correlation values for the iButtonTM, water-table depth and stable isotope data for *Sphagnum* (C, O and H isotopes), plant-associated

waters (O, H) and deuterium excess (d). Isotope, relative humidity and temperature data are presented.

Figure S1. Composite correlation diagram for deuterium excess (d) and water-table depth against temperature and relative humidity.

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Abbreviations. LMWL, local meteoric water line.

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