University of Wollongong

Research Online

Faculty of Science, Medicine and Health -
Papers: part A

Faculty of Science, Medicine and Health

1-1-2015

Moss $\delta^{13}C$: an accurate proxy for past water environments in polar regions

Jessica Bramley-Alves

University of Wollongong, jba605@uowmail.edu.au

Wolfgang Wanek

University of Vienna, jane.wasley@aad.gov.au

Kris French

University of Wollongong, kris@uow.edu.au

Sharon A. Robinson

University of Wollongong, sharonr@uow.edu.au

Follow this and additional works at: <https://ro.uow.edu.au/smhpapers>

Part of the [Medicine and Health Sciences Commons](#), and the [Social and Behavioral Sciences Commons](#)

Recommended Citation

Bramley-Alves, Jessica; Wanek, Wolfgang; French, Kris; and Robinson, Sharon A., "Moss $\delta^{13}C$: an accurate proxy for past water environments in polar regions" (2015). *Faculty of Science, Medicine and Health - Papers: part A*. 2757.

<https://ro.uow.edu.au/smhpapers/2757>

Research Online is the open access institutional repository for the University of Wollongong. For further information contact the UOW Library: research-pubs@uow.edu.au

Moss $\delta^{13}\text{C}$: an accurate proxy for past water environments in polar regions

Abstract

Increased aridity is of global concern. Polar regions provide an opportunity to monitor changes in bioavailable water free of local anthropogenic influences. However, sophisticated proxy measures are needed. We explored the possibility of using stable carbon isotopes in segments of moss as a fine-scale proxy for past bioavailable water. Variation in $\delta^{13}\text{C}$ with water availability was measured in three species across three peninsulas in the Windmill Islands, East Antarctica and verified using controlled chamber experiments. The $\delta^{13}\text{C}$ from Antarctic mosses accurately recorded long-term variations in water availability in the field, regardless of location, but significant disparities in $\delta^{13}\text{C}$ between species indicated some make more sensitive proxies. $\delta^{13}\text{C}_{\text{SUGAR}}$ derived from living tissues can change significantly within the span of an Antarctic season (5 weeks) in chambers, but under field conditions, slow growth means that this technique likely represents multiple seasons. $\delta^{13}\text{C}_{\text{CELLULOSE}}$ provides a precise and direct proxy for bioavailable water, allowing reconstructions for coastal Antarctica and potentially other cold regions over past centuries.

Keywords

regions, proxy, past, accurate, water, ^{13}C , moss, environments, polar

Disciplines

Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details

Bramley-Alves, J., Wanek, W., French, K. & Robinson, S. A. (2015). Moss $\delta^{13}\text{C}$: an accurate proxy for past water environments in polar regions. *Global Change Biology*, 21 (6), 2454-2464.

1 **Moss $\delta^{13}\text{C}$: an accurate proxy for past water environments in polar regions**

2 **Running Head:** Moss $\delta^{13}\text{C}$ is a proxy for bio-available water

3 Jessica Bramley-Alves¹, Wolfgang Wanek², Kristine French¹ and Sharon A. Robinson¹

4 ¹Institute for Conservation Biology, School of Biological Sciences, University of

5 Wollongong, New South Wales 2522 Australia.

6 ²Faculty of Life Sciences, Department of Microbiology and Ecosystem Science, University of

7 Vienna, Austria.

8 **Corresponding author:** Jessica Bramley-Alves, T +61 4 8767 6295, jba605@gmail.com

9 **Keywords:** $\delta^{13}\text{C}$, Antarctica, cellulose, cell wall thickness, climate change, proxies, bio-
10 available water.

11 **Technical Advances**

Abstract

Increased aridity is of global concern. Polar regions provide an opportunity to monitor changes in bio-available water free of local anthropogenic influences. However, sophisticated proxy measures are needed. We explored the possibility of using stable carbon isotopes in segments of moss as a fine-scale proxy for past bio-available water. Variation in $\delta^{13}\text{C}$ with water availability was measured in three species across three peninsulas in the Windmill Islands, East Antarctica and verified using controlled chamber experiments. The $\delta^{13}\text{C}$ from Antarctic mosses accurately recorded long-term variations in water availability in the field, regardless of location, but significant disparities in $\delta^{13}\text{C}$ between species indicated some make more sensitive proxies. $\delta^{13}\text{C}_{\text{SUGAR}}$ derived from living tissues can change significantly within the span of an Antarctic season (5 weeks) in chambers, but under field conditions, slow growth means that this technique likely represents multiple seasons. $\delta^{13}\text{C}_{\text{CELLULOSE}}$ provides a precise and direct proxy for bio-available water, allowing reconstructions for coastal Antarctica and potentially other cold regions over past centuries.

Introduction

The impact of climate change on bio-available water, and its subsequent ecological effect, is of considerable concern (IPCC, 2007; van Ommen & Morgan, 2010; IPCC, 2014). Polar regions are important locations in assessing such change as their isolation provides a clear climate change response signal relatively independent of confounding anthropogenic influences (Callaghan *et al.*, 1992; Turner *et al.*, 2002). Additionally, the simplicity of ecological systems in these regions means that they are likely to respond rapidly to climate variations, making them ideal baseline environments for the study of global change (Bergstrom & Chown, 1999; Robinson *et al.*, 2003; Lebouvier *et al.*, 2011).

1 Our current understanding of changes to bio-available water in these regions is limited by our
2 dependence on either long-term proxy based information from ice cores (Mayewski *et al.*,
3 2009; Matsumoto *et al.*, 2011), or short-term, and spatially disparate, meteorological records
4 (Fig. 1). Since bio-available water depends on a balance between a multitude of interacting
5 inputs such as precipitation (snow) and meltwater from permanent ice banks (Lucieer *et al.*,
6 2014) and outputs (including loss as sublimation, run off and evaporation), which are dictated
7 by climatic factors such as temperature and wind speed (Clarke *et al.*, 2012; Convey *et al.*,
8 2014; Robinson & Erickson, 2014), characterising bio-available water based purely on
9 meteorological data is arguably inadequate.

10 Stable carbon isotopes ($\delta^{13}\text{C}$) within Antarctic bryophytes offer a potential integrated measure
11 of bio-available water as a result of RuBisCOs preferential fixation of the lighter ^{12}C isotope
12 during photosynthesis (Farquhar *et al.*, 1989; Rice & Giles, 1996). Unlike vascular plants,
13 bryophytes lack stomata and as a result cannot regulate their carbon dioxide or water uptake
14 physiologically (Rice & Giles, 1996; Williams & Flanagan, 1996; Rice, 2000). Accordingly,
15 the resistance to CO_2 diffusion in moss is divided into two extreme environmental conditions:
16 either when the leaf surface is dry or, when the surface is wet or submerged in water (Rice &
17 Giles, 1996). In the latter condition, the presence of a water film can create large barriers to
18 carbon assimilation due to the much lower CO_2 diffusivity in water than in air (Rice & Giles,
19 1996). Hence, $\delta^{13}\text{C}$ values are less negative (less discrimination against isotopically-heavier
20 $^{13}\text{CO}_2$) when plants are covered by diffusion-limiting water films. Conversely, $\delta^{13}\text{C}$ values are
21 more negative when plants are drier and diffusion and photosynthetic rates optimized, as
22 RuBisCO consistently favours the isotopically-lighter $^{12}\text{CO}_2$ molecules (Rice, 2000; Royles &
23 Griffiths, 2014).

1 Mosses are widely distributed in coastal regions of Antarctica and, due to the severity of
2 Antarctic conditions, have very slow growth rates relative to moss in more temperate regions
3 ($<6 \text{ mm yr}^{-1}$; Convey *et al.*, 2014) giving their records the potential to span centuries. Mosses
4 lack vascular tissue and grow sequentially from the tip on a seasonal basis, with each new
5 section capturing and sequestering its own photosynthetic carbon. Like tree rings, each
6 section thus captures a snapshot of environmental growth conditions. Difficulties experienced
7 when using vascular plants as long-term climate proxies can be avoided when using non-
8 vascular species, such as mosses. For example, $\delta^{13}\text{C}$ in vascular plants is influenced by
9 different $\delta^{13}\text{C}$ signatures in the various chemicals that make up the vascular system (Benner *et*
10 *al.*, 1987; Robinson *et al.*, 1993). In addition, differential breakdown of these various
11 constituents over time further complicates interpretations; lignin breaks down more slowly
12 than polysaccharides, causing shifts in $\delta^{13}\text{C}$ values by up to 4‰ (Benner *et al.*, 1987; Schleser
13 *et al.*, 1999; McCarroll & Loader, 2004). Unlike vascular plants mosses have a reduced
14 proportion of lignin (Weng & Chapple, 2010), due to the simplicity of their hydrological
15 systems, and consist largely of cellulose (43%; Skrzypek *et al.*, 2007). This makes mosses an
16 ideal candidate for the long-term study of past climate, especially in Antarctica where cold
17 conditions further preserve compounds over time (Royles *et al.*, 2013; Roads *et al.*, 2014) and
18 lateral transfer of depleted ^{13}C into mosses is unlikely due to the low levels of inorganic and
19 organic carbon in the environment (Sobek *et al.*, 2007; Sawstrom *et al.*, 2008).

20 Novel advances in precision dating down intact moss shoots via the ^{14}C ‘bomb pulse’ have
21 allowed high resolution exploration of climate change in East Antarctica (Clarke *et al.*, 2012)
22 and more recently in the Maritime Antarctic (Royles *et al.*, 2012; Royles *et al.*, 2013). These
23 studies all used $\delta^{13}\text{C}$ as an indication of past bio-available water. However, until we have
24 substantiated how $\delta^{13}\text{C}$ signatures in moss species vary under their existing growth conditions
25 current and future reconstructions of water environments using $\delta^{13}\text{C}$ in Antarctic moss cores

1 are unreliable. Whilst the use of $\delta^{13}\text{C}$ in non-vascular plants has been supported as a long-
2 term proxy for growth water in temperate locations (Menot-Combes *et al.*, 2004; Charman *et*
3 *al.*, 2009; Booth *et al.*, 2010; Kaislahti Tillman *et al.*, 2010; Yamamoto *et al.*, 2010) no one,
4 to the best of our knowledge, has validated the capacity of $\delta^{13}\text{C}$ signatures in Antarctic moss
5 species to accurately reflect bio-accessible water. This is important as discrimination against
6 $^{13}\text{CO}_2$ may vary across environmental gradients, climates and between species (O'Leary,
7 1981; Farquhar *et al.*, 1982; Lajtha & Getz, 1993; Hultine & Marshall, 2000; Dawson *et al.*,
8 2002; Sah & Brumme, 2003; Menot-Combes *et al.*, 2004; Royles *et al.*, 2014). For example,
9 morphological traits - such as cell wall thickness - may differ between growth environments
10 and thus influence the assimilation of carbon into cells (Waite & Sack, 2010). Likewise, plant
11 growth form may directly or indirectly influence diffusional resistance in cells by either
12 altering boundary layers surrounding plant surfaces (Rice & Schuepp, 1995), or affecting the
13 development of surface water film, respectively. Significant species-specific $\delta^{13}\text{C}$ variations
14 have already been documented (Zhang *et al.*, 1993; Pancost *et al.*, 2003; Menot-Combes *et*
15 *al.*, 2004; Loader *et al.*, 2007; Moschen *et al.*, 2009; Brader *et al.*, 2010) especially in
16 Antarctic mosses (Galimov, 2000; Wasley *et al.*, 2006). Therefore, $\delta^{13}\text{C}$ analysis in Antarctic
17 moss cores using more than one species, or a species that is not a sensitive recorder of growth
18 water environment, may be open to misinterpretation.

19 We sought to explore quantitatively the factors that influence $\delta^{13}\text{C}$ in Antarctic species and
20 environments in order to validate $\delta^{13}\text{C}$ as a paleohydrological proxy for this region. Our
21 primary objective was to enable interpretation of past Antarctic water trends using $\delta^{13}\text{C}$ in
22 long intact shoots of moss. Correlating such water trends to climatic drivers would expand our
23 knowledge of the effect of current climate change in the region (Clarke *et al.*, 2012) and
24 potentially other areas of the world (van Ommen & Morgan, 2010). The capacity to
25 distinguish intra-seasonal shifts in Antarctic growth water might also be possible through

analysis of moss soluble carbohydrates ($\delta^{13}\text{C}_{\text{SUGAR}}$). While $\delta^{13}\text{C}_{\text{SUGAR}}$ may not be as useful in paleo-research, as compounds could be leached and lost as cells dry, sugars have been found to display environmental fluctuations within hours to days (Brugnoli *et al.*, 1988) and therefore could theoretically detect subtle shifts in water availability within single growing seasons. Completely new wall material (cellulose), on the other hand, takes longer to establish and initially is diluted with the old cellulose signatures. Specifically we examine: (1) the influence of growth water environment on $\delta^{13}\text{C}$ in Antarctic moss under laboratory and field conditions; (2) inter-species variability and (3) the temporal resolution with which $\delta^{13}\text{C}_{\text{CELLULOSE}}$ and $\delta^{13}\text{C}_{\text{SUGAR}}$ compounds can detect change. Finally, we use our findings to show changes in growth water availability in East Antarctic moss shoots using $\delta^{13}\text{C}_{\text{GRAPHITE}}$.

Materials and methods

Study sites and species

Three moss species, two cosmopolitan, *Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer & Scherb. and *Ceratodon purpureus* (Hedw.) Brid., and the endemic *Schistidium antarctici* Card., were collected from three peninsulas (Clark and Bailey peninsulas and Robinson Ridge; S1-3 respectively in Fig. 1) in the Windmill Islands (66°15'S, 110°33'E), East Antarctica (detailed description in Wasley *et al.*, 2006).

Field investigations

Moss plugs ($\sim 2 \text{ cm}^2$) of each species were collected on each peninsula (Fig. 1) from hydric areas where moss is known to remain submerged throughout the season, xeric areas where moss relies on ephemeral water sources such as snowfall and mesic areas in a transitional water environment ($n = 7-10$; see Wasley *et al.*, (2012) for detailed water gradient description).

To allow spot measurements of moss turf water content (TWC; Lucieer *et al.*, 2014) to be compared with the visual estimates of long-term water environments and $\delta^{13}\text{C}$, moss plugs (18 *C. purpureus*) were also sampled from established wet, intermediate and dry study sites across two permanent, long-term monitoring sites: Antarctic Specially Protected Area (ASPA) 135 on Bailey Peninsula and Robinson Ridge (S2-3 respectively, Fig. 1). These sites form State of the Environment Indicator 72 (http://aadcmaps.aad.gov.au/aadc/soe/display_indicator.cfm?soe_id=72).

Growth chamber manipulations

A five-week pilot study was conducted (January - February 2012) to evaluate if $\delta^{13}\text{C}_{\text{CELLULOSE}}$ and $\delta^{13}\text{C}_{\text{SUGAR}}$ in Antarctic moss varied in response to changing water environments within a single Antarctic growth season. Five weeks was deemed ample time to generate sufficient new growth for analysis based on similar chamber studies that demonstrated growth rates of 9.87 ± 0.83 mm for *B. pseudotriquetrum* (Supporting Information, Fig. S3 a & e) and 5.17 ± 0.39 mm for *C. purpureus* (Bramley-Alves, unpublished data). Plugs (depth ~1 cm) of *C. purpureus*, *S. antarctici* and *B. pseudotriquetrum* (n=24) were collected from a range of water environments on Bailey peninsula (Fig. 1, S2). Samples were placed in microplates (24 well, Corning, Australia) and randomly allocated to one of three water treatments within growth chambers in the science laboratory at Casey station. The treatments represented three different environmental conditions: wet; where samples were kept submerged under more than 3 mm of water, intermediate; where samples were not submerged but were provided with an ample water supply, or dry; where samples were given the minimum level of water to allow growth ($> 2 \text{ g H}_2\text{O g}^{-1}$ Dry Weight; Wasley *et al.*, 2006) and were never inundated. To avoid formation of a water film, dry samples were watered at the base of the moss core via a Pasteur pipette. Chambers were kept at a constant 15°C with a natural summer photoperiod

(22 h $\sim 700 \text{ umols m}^{-2} \text{ s}^{-1}$ PAR) to mirror moss turf conditions in the field during the summer growth period, where turf temperatures can reach $> 20^\circ\text{C}$ (Bramley-Alves *et al.*, 2014). Chamber relative humidity was $> 60\%$ (Kestrel 3500, Delta T, USA).

A simplified version of the growth chamber experiment was repeated in Australia in 2013 but run for a longer time interval (22 weeks) to explore inter-seasonal shifts in $\delta^{13}\text{C}$. All three species ($n=16$) were collected as above, air dried to a constant mass and frozen for transport back to Australia. Once rehydrated (over 24 h) samples from each species were randomly allocated to one of two water treatments (dry or wet) in growth cabinets at the University of Wollongong, Australia. Water treatments and chamber conditions were as described above.

Sample preparation

Samples were oven dried to a constant mass at 60°C . As atmospheric CO_2 concentrations have remained relatively constant over the past 5 years (Rubino *et al.*, 2013), 3 mm sections (integrating 2.3 to 5 years of growth based on growth-rates from Clarke *et al.*, (2012)) were cut from the moss plugs collected from the field, whilst exclusively new growth material was cut from moss grown under chamber conditions ($\sim 2\text{-}4 \text{ mm}$). All samples were ground to a fine powder in a ball mill TissueLyser (Qiagen, Retsch, Australia) at 1600 g for 3 minutes. Between 1.5 - 2 mg of whole moss powder was weighed directly into tin capsules (5.25 mm x 3.2 mm) and sealed for $\delta^{13}\text{C}_{\text{BULK}}$ analysis.

Soluble carbohydrates were isolated after Wanek *et al.*, (2001) and cellulose was extracted after Hietz *et al.*, (2005). Carbon isotope analyses were conducted at the University of Vienna on a continuous-flow Isotopic Ratio Mass Spectrometer (IRMS Delta^{PLUS}, Finnigan MAT) interfaced to an Elemental Analyzer (EA 1110, CE Instrument, Milan, Italy) by ConFlo II

(Finnigan MAT, Bremen, Germany). The abundance of ^{13}C relative to ^{12}C in samples was calculated by the following equation:

$$\delta^{13}\text{C} (\text{‰}) = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right)$$

where R is the ratio of mass ^{13}C to mass ^{12}C . The $\delta^{13}\text{C}$ value of samples are reported relative to Vienna PeeDee Belemnite (V-PDB) standards and expressed as parts ‘per mil’ (‰). Samples were measured against a CO_2 reference gas, calibrated to IAEA-CH-6 and IAEA-CH-7 reference material (International Atomic Energy Agency, Vienna, Austria). A typical standard deviation of $\delta^{13}\text{C}$ analysis for replicate samples is $\pm 0.1\text{‰}$.

Temporal changes

We used data from three previously dated *C. purpureus* cores (Clarke *et al.*, 2012) in order to interpret past trends in Antarctic water environments in the context of our current $\delta^{13}\text{C}$ findings. Conventional ^{14}C ages and $\delta^{13}\text{C}_{\text{GRAPHITE}}$ (a bi-product of the ^{14}C dating process, see Hua *et al.* (2001) for further details) were remodeled using the OxCal v4.0 program (Bronk Ramsey, 1995, 2001). Changes to atmospheric CO_2 influence core $\delta^{13}\text{C}$ through time (Francey *et al.*, 1999) and recent updates for atmospheric CO_2 values (Rubino *et al.*, 2013) could thus be incorporated into models.

Statistical analysis

The change in $\delta^{13}\text{C}$ between wet and dry environments ($\text{WD}\delta^{13}\text{C}$) was calculated by subtracting the mean dry $\delta^{13}\text{C}$ from the mean wet $\delta^{13}\text{C}$. All data analyses were conducted in the statistical program JMP (Ver. 5.1 SAS Institute Inc., U.S.). Prior to analysis, raw data were tested for normality using the Shapiro-Wilks W Test, and for homogeneity of variance using Cochran’s C test. Transformations were performed when necessary to satisfy

assumptions. Data were analysed using general linear models with a two-way ANOVA or ANCOVA, with environment and species as the main effects alongside their interaction term. Where significant interactions were found, Tukey HSD *post hoc* tests were used to identify significantly different means between multiple groups. Students-t tests were used for comparisons between two groups.

Results

A strong relationship ($R^2 = 0.98$) was found between $\delta^{13}\text{C}_{\text{BULK}}$ and $\delta^{13}\text{C}_{\text{CELLULOSE}}$ in Antarctic *C. purpureus* (Supporting Information, S1). Our work confirms that both materials are suitable for paleo-climatic research, especially under cold Antarctic conditions which further preserve compounds over time (Menot & Burns, 2001; Skrzypek *et al.*, 2007; Royles, 2012).

Field investigations

$\delta^{13}\text{C}_{\text{BULK}}$ differed significantly ($F_{2,4} = 13.78$, $p = 0.016$) between water environments in the field (Fig. 2), regardless of collection location. Wet sites were significantly less negative ($-24.4 \pm 1.8\text{‰}$) than either intermediate ($-25.2 \pm 1.3\text{‰}$) or dry ($-26.0 \pm 1.1\text{‰}$) sites across all locations and for all species, with a mean difference of 1.6‰ between dry and wet sites. $\delta^{13}\text{C}_{\text{BULK}}$ also varied significantly between each of the three study species ($F_{2,4} = 28.82$, $p = 0.004$) with *S. antarctici* displaying the least negative values ($-24.2 \pm 1.5\text{‰}$), *B. pseudotriquetrum* the most negative ($-26.0 \pm 1.2\text{‰}$) and *C. purpureus* intermediate values between the two ($-25.2 \pm 1.6\text{‰}$). $\text{WD}\delta^{13}\text{C}_{\text{BULK}}$ between environments was greatest in *C. purpureus* (2.1‰), followed by *S. antarctici* (1.5‰) and *B. pseudotriquetrum* (1.0‰).

The relationship between *C. purpureus* $\delta^{13}\text{C}_{\text{CELLULOSE}}$ and TWC (Fig. 3), collected from established wet, intermediate or dry locations within two permanent long-term monitoring sites, was significant ($p < 0.0007$, $R^2 = 0.44$).

Controlled manipulations

Moss $\delta^{13}\text{C}_{\text{SUGAR}}$ revealed a significant interaction between species and environment ($F_{8, 54} = 3.51$, $p = 0.013$) within 5 weeks of growth under the different water treatments (Fig. 4). All three species displayed $\delta^{13}\text{C}_{\text{SUGAR}}$ values that were less negative in wet than in dry environments but this difference was not significant for *S. antarctici*. For *B. pseudotriquetrum* the wet treatment $\delta^{13}\text{C}_{\text{SUGAR}}$ was significantly less negative than that in the intermediate treatment, while in *C. purpureus* the intermediate treatment $\delta^{13}\text{C}_{\text{SUGAR}}$ was between, and similar, to both wet and dry treatments. Intermediate and dry $\delta^{13}\text{C}_{\text{SUGAR}}$ did not show significant differences for any species. When comparing species, $\delta^{13}\text{C}_{\text{SUGAR}}$ for both *C. purpureus* and *B. pseudotriquetrum* were more negative than for *S. antarctici*. $\text{WD}\delta^{13}\text{C}_{\text{SUGAR}}$ was similar for all species, ranging from 1 - 1.6‰ (Table 1).

Moss $\delta^{13}\text{C}_{\text{CELLULOSE}}$ also differed significantly between water treatments within 5 weeks ($F_{8, 54} = 6.41$, $p = 0.0032$; Table 2). Intermediate treatment $\delta^{13}\text{C}_{\text{CELLULOSE}}$ ($-19.2 \pm 1.49\text{‰}$) was significantly less negative than dry treatment $\delta^{13}\text{C}_{\text{CELLULOSE}}$ ($-20.7 \pm 1.34\text{‰}$) with wet treatment $\delta^{13}\text{C}_{\text{CELLULOSE}}$ intermediate ($-20.2 \pm 1.66\text{‰}$). $\delta^{13}\text{C}_{\text{CELLULOSE}}$ also differed significantly between species ($F_{8, 54} = 7.56$, $p = 0.0013$), with *S. antarctici* displaying significantly less negative $\delta^{13}\text{C}_{\text{CELLULOSE}}$ ($-19.2 \pm 1.32\text{‰}$) than either *C. purpureus* ($-20.7 \pm 1.60\text{‰}$) or *B. pseudotriquetrum* ($-20.2 \pm 1.50\text{‰}$), which were statistically similar. $\text{WD}\delta^{13}\text{C}_{\text{CELLULOSE}}$ between environments was highest for *B. pseudotriquetrum* but was negative for *C. purpureus* (Table 1).

Moss $\delta^{13}\text{C}_{\text{SUGAR}}$ differed significantly between water treatments after 22 weeks ($F_{5, 40} = 20.86$, $p = 0.0001$; Fig. 5a). Inundation resulted in significantly less negative $\delta^{13}\text{C}_{\text{SUGAR}}$, ranging from -24.3 to -20.1‰ compared to -27.0 to -21.1‰ under dry treatments, with a mean difference of 1.8‰. $\delta^{13}\text{C}_{\text{SUGAR}}$ also varied significantly between species ($F_{5, 40} = 4.17$, $p =$

0.022; Fig. 5b), with *S. antarctici* displaying significantly less negative $\delta^{13}\text{C}_{\text{SUGAR}}$ than *C. purpureus*. $\text{WD}\delta^{13}\text{C}_{\text{SUGAR}}$ between water treatments was much greater in *C. purpureus* than in *B. pseudotriquetrum* or *S. antarctici* (Table 1).

Moss $\delta^{13}\text{C}_{\text{CELLULOSE}}$ also differed significantly between water treatments after 22 weeks ($F_{5,39} = 9.40$, $p = 0.0039$), with moss $\delta^{13}\text{C}_{\text{CELLULOSE}}$ values 1.7‰ less negative under inundated conditions ($-21.69 \pm 1.81\text{‰}$) than when grown under dry conditions ($-23.45 \pm 2.09\text{‰}$; Fig. 5c). $\delta^{13}\text{C}_{\text{CELLULOSE}}$ did not vary significantly between species (Fig. 5d). $\text{WD}\delta^{13}\text{C}$ between environments was again highest in *C. purpureus*, followed by *B. pseudotriquetrum* with *S. antarctici* the least different (Table 1).

$\delta^{13}\text{C}_{\text{CELLULOSE}}$ was consistently more negative than $\delta^{13}\text{C}_{\text{SUGAR}}$ in Antarctic moss, with an offset of 0.9‰. The relationship between $\delta^{13}\text{C}_{\text{CELLULOSE}}$ and $\delta^{13}\text{C}_{\text{SUGAR}}$ compounds was not significant after 5 weeks of growth (Fig. 6a). Significant differences in signatures were seen among species ($F_{5,56} = 3.35$, $p = 0.04$). However, $\delta^{13}\text{C}_{\text{CELLULOSE}}$ was positively correlated with $\delta^{13}\text{C}_{\text{SUGAR}}$ samples after 22 weeks ($R^2 = 0.6$; Fig. 6b) with no significant differences among species ($F_{5,39} = 1.45$, $p = 0.24$).

Discussion

Our results demonstrate that Antarctic mosses consistently show significantly less negative $\delta^{13}\text{C}$ values in wet environments in the field (Fig. 2), regardless of collection location; a result confirmed in these species under laboratory conditions (Fig. 4 & 5). $\delta^{13}\text{C}_{\text{SUGAR}}$ in these species can capture intra-seasonal shifts, but under Antarctic field conditions likely represent inter-season trends. This information can now be used to accurately interpret past Antarctic water trends using $\delta^{13}\text{C}$ in long intact shoots of moss.

Inter-species $\delta^{13}\text{C}$ variability

1 While $\delta^{13}\text{C}$ accurately represented water environments across all study species, the $\delta^{13}\text{C}$ range
2 of each species differed significantly (Fig. 2). This highlights the need to use single species
3 when interpreting paleo-environmental information down a core or to examine the differences
4 between species prior to interpretation. For example, $\delta^{13}\text{C}$ values of *S. antarctici* were 1‰
5 and 1.8‰ less negative than *C. purpureus* and *B. pseudotriquetrum* under field conditions.
6 Whilst small, this difference is highly significant: shifts of 0.9‰ can underline differences
7 between wet and intermediate environments in Antarctica and shifts of 1.5‰ can depict
8 differences between wet and dry environments (c.f. Fig. 2; 3; 4; 5). The significance is
9 likewise demonstrated in studies that linked shifts between -0.4 and +0.4‰ in a peat bog to a
10 1°C change in temperature (Menot & Burns, 2001). For example, using the data in Fig. 2, if
11 $\delta^{13}\text{C}_{\text{BULK}}$ values in samples from a mixed or unidentified species core were interpreted and a
12 $\delta^{13}\text{C}_{\text{BULK}}$ of -25.5‰ obtained, it could be concluded this was either a wet environment for
13 *B. pseudotriquetrum*, an intermediate environment for *C. purpureus* or a dry environment for
14 *S. antarctici*. As a result, the use of conspecific intact moss shoots represents a more accurate
15 technique for elucidating past Antarctic water environments than interpreting changes down
16 heterospecific peat cores. Greater knowledge of the species composition of peat cores and
17 their isotopic range will provide a far more accurate interpretation of water availability during
18 growing seasons.

19 The significant $\delta^{13}\text{C}$ variation between species grown under identical conditions implies that
20 there are physiological differences between the species that inherently influence $\delta^{13}\text{C}$. We
21 suggest that these differences may be controlled by either leaf morphology or plant growth
22 form. In some moss species, for example *Sphagnum* and *C. purpureus*, water-filled hyaline
23 cells (Stanton *et al.*, 2014) control diffusion into photosynthetic cells and therefore influence
24 diffusion resistance – a feature that has been proposed to explain variation in $\delta^{13}\text{C}$ in
25 *Sphagnum* species (Menot & Burns, 2001). Thick cell walls, which act like the viscous barrier

1 of water, may also constrain carbon assimilation into cells. For example, branch dwelling
2 moss species were found to have thicker cell walls than ground dwelling species,
3 corresponding to lower carbon assimilation per mass in branch dwelling species (Waite &
4 Sack, 2010). The ranking of $\delta^{13}\text{C}$ values for the moss species in this study suggest that cell
5 walls may affect diffusion and therefore discrimination; with *S. antarctici* displaying the
6 thickest cell walls and least negative $\delta^{13}\text{C}$ values and *C. purpureus* and *B. pseudotriquetrum*
7 the thinnest walls and most negative $\delta^{13}\text{C}$ values (Supporting Information, Fig. S2). Cell wall
8 thickness was constant across water environments in the field for *C. purpureus* and
9 *B. pseudotriquetrum* suggesting that the higher $\delta^{13}\text{C}$ values displayed in wet environments
10 were independent of morphological changes. Interestingly, continual submergence has been
11 found to produce thinner boundary layers in *Sphagnum* species, which in turn may reduce
12 resistance to CO_2 diffusion into the cell (Rice & Schuepp, 1995). We found that *S. antarctici*
13 generated thinner cell walls under wet compared to dry growth conditions, implying there
14 should be greater diffusional resistance (and less negative $\delta^{13}\text{C}$) in dry environments (the
15 opposite of that found). This means that the less negative $\delta^{13}\text{C}$ values seen in *S. antarctici*
16 under wet conditions is predominantly related to diffusional restrictions by the surrounding
17 water and must be sufficiently large to overcome any increased diffusion of CO_2 due to
18 thinner walls.

19 Growth form could also influence $\delta^{13}\text{C}$ signatures among species. Unlike *S. antarctici* and
20 *C. purpureus*, which grow as smooth, uniform moss turfs, *B. pseudotriquetrum* gametophytes
21 often grow through and above the surrounding turf. This growth form means that
22 photosynthetically active tissue likely avoids submergence in environments where the other
23 two species would be inundated, potentially giving *B. pseudotriquetrum* a more negative $\delta^{13}\text{C}$
24 signature regardless of its micro-environment. A five-week chamber study simulating growth
25 under moss cover showed gametophytes of *B. pseudotriquetrum* etiolate rapidly when faced

1 with limited light (conditions similar to a packed moss turf, Supporting Information, S3).
2 Stem length was over 4x that of the control when moss was placed under 50% simulated moss
3 cover. When this growth form was controlled under laboratory conditions, by keeping
4 *B. pseudotriquetrum* inundated for five weeks, $\delta^{13}\text{C}_{\text{SUGAR}}$ in *B. pseudotriquetrum* was similar
5 to that in *C. purpureus*, and only different to *S. antarctici* (c.f. with field experiment Fig. 2).
6 Similarly, when the experiment was extended to 22 weeks all species showed similar
7 $\delta^{13}\text{C}_{\text{CELLULOSE}}$ with only water treatments producing significantly different $\delta^{13}\text{C}_{\text{CELLULOSE}}$.
8 These findings suggest that under natural conditions *B. pseudotriquetrum*'s emergent growth
9 form contributes to its more negative $\delta^{13}\text{C}$ signatures.

10 This inundation avoidance response by *B. pseudotriquetrum* means that it is a less useful
11 proxy for surrounding water conditions, despite its relatively large change in $\delta^{13}\text{C}$ between
12 wet and dry environments under controlled conditions. While morphological changes in
13 *S. antarctici* have the potential to counteract the trend toward greater diffusional limitation
14 under inundated conditions our results show that it is still an accurate recorder of micro-site
15 water environment. Of the three species *C. purpureus* seems physiologically best suited to
16 capturing changes in water environments ($\text{WD}\delta^{13}\text{C} > 3\text{‰}$ in the field and after 22 weeks in
17 the laboratory) provided it is given adequate time to grow (> 5 weeks).

18 *Temporal and environmental resolution*

19 Variations in growth water conditions were distinguishable in significantly less negative
20 $\delta^{13}\text{C}_{\text{SUGAR}}$ signals of *B. pseudotriquetrum* and *C. purpureus* within just five weeks of growth
21 under inundated conditions inside chambers. This shows that these species have the potential
22 to record water variation within a short time frame, with $\delta^{13}\text{C}_{\text{SUGAR}}$ capturing changes across
23 the span of a typical Antarctic growth season. After 22 weeks $\delta^{13}\text{C}_{\text{SUGAR}}$ and $\delta^{13}\text{C}_{\text{CELLULOSE}}$
24 were well correlated (Fig. 6b), which was not the case after 5 weeks (Fig. 6a) suggesting that

1 whilst sugars quickly incorporated new carbon, this was not reflected fully in new wall
2 material until several months later (due to the slower dilution of old with newly formed
3 cellulose). Thus $\delta^{13}\text{C}_{\text{CELLULOSE}}$ and $\delta^{13}\text{C}_{\text{BULK}}$ provide better proxies for longer term, inter-
4 seasonal changes. It should be recognized that chamber studies provide optimal conditions for
5 moss growth and do not represent the severity of the Antarctic climate. In addition to
6 changing water availability Antarctic mosses deal with fluctuating diel temperatures (from -9
7 to 30°C at the turf surface, Bramley-Alves *et al.*, 2014), periods of freeze-thaw, highly
8 variable PAR and high UV radiation. These can all inhibit growth resulting in slow
9 accumulation of new tissue and subsequent changes in moss $\delta^{13}\text{C}$. This is illustrated in the
10 results of a reciprocal transplant field experiment, where $\delta^{13}\text{C}_{\text{SUGAR}}$ signatures were similar to
11 initial controls after five weeks (Supporting Information, S4). Field $\delta^{13}\text{C}$ signatures take a
12 relatively long time to establish, due to the short Antarctic summers, and provide an overall
13 assessment of longer-term (3-5 year) water variability rather than a snapshot of wet or dry
14 periods within a season. In terms of gaining long-term information about past climate this
15 serves our purpose well. However, our research suggests that intra-seasonal shifts in growth
16 water could be assessed through $\delta^{13}\text{C}_{\text{SUGAR}}$ under less stressful conditions such as the
17 maritime Antarctic (see Royles & Griffiths, 2014).

18 Our findings allow us to quantitatively assess variation in $\delta^{13}\text{C}$ across temporal scales for the
19 first time (Fig. 7). Changes in $\delta^{13}\text{C}_{\text{GRAPHITE}}$ down *C. purpureus* cores previously dated by
20 Clarke *et al.*, (2012) suggested that sites in the Windmill Islands had dried over recent
21 decades, but gave no sense of the scale of these changes. Using the $\delta^{13}\text{C}$ ranges established
22 for this species in Fig. 2 we can now confirm that prior to the 1970s these sites would have
23 been classified as wet-intermediate, whereas from the 1990s onward they would be classed as
24 dry-intermediate. In addition, the correlation between spot TWC and $\delta^{13}\text{C}$ (Fig. 3) suggests
25 that the water availability has declined by approximately half between 1980 and 2000. This

1 regional drying has been linked to changing wind patterns as a result of ozone depletion
2 (Robinson & Erickson, 2014).

3 We propose that tracing carbon isotopes down long shoots of dated moss is a viable and
4 valuable proxy for assessing past water environments in coastal areas of Antarctica that have
5 limited climate information. However, significant disparities in $\delta^{13}\text{C}$ exist among species and
6 therefore reconstructions based on multiple species should be avoided. Whilst $\delta^{13}\text{C}_{\text{SUGAR}}$ has
7 the potential to show rapid changes within a season of growth under laboratory conditions or
8 in the maritime Antarctic, $\delta^{13}\text{C}_{\text{BULK}}$ or $\delta^{13}\text{C}_{\text{CELLULOSE}}$ (especially if degradation is suspected)
9 reveal inter-seasonal changes in the field in continental Antarctica. Moss $\delta^{13}\text{C}$ represents an
10 effective integrator of growth water and could resolve past histories and spatial variation over
11 large sections of Antarctica where we currently have no information – increasing our ability
12 to understand the past and present, and so to forecast the future.

13 **Acknowledgements**

14 This research was funded through AINSE05142P/06155, ARC DP110101714 and AAS
15 3129/4046 grants. JBA held an Australia Postgraduate Award during the time of the study.
16 We thank Melinda Waterman for remodeling ^{14}C ages, Margarete Watzka for assistance with
17 isotope analysis, the Robinson Research Group, Guy Norton and anonymous reviewers for
18 improving the manuscript and Andrew Netherwood for preparing Fig. 1 and 7.

19 **References**

20 Australian Antarctic Division (2012) Map A: Antarctic Specially Protected Areas, Windmill
21 Islands, East Antarctica. No. 14197, Australian Antarctic Division, Hobart.

- 1 Benner R, Fogel ML, Sprague EK, Hodson RE (1987) Depletion of $\delta^{13}\text{C}$ in lignin and its
2 implications for stable carbon isotope studies. *Nature*, **329**, 708-710.
- 3
- 4 Bergstrom DM, Chown S (1999) Life at the front: history, ecology and change on southern
5 ocean islands. *TREE*, **14**, 472-477.
- 6
- 7 Booth RK, Jackson ST, Notaro M (2010) Using peatland archives to test paleoclimate
8 hypotheses. *PAGES news*, **18**, 6 - 8.
- 9
- 10 Brader AV, van Winden JF, Bohncke SJP, Beets CJ, Reichert G, de Leeuw J (2010)
11 Fractionation of hydrogen, oxygen and carbon isotopes in n-alkanes and cellulose of three
12 *Sphagnum* species. *Organic Geochemistry*, **41**, 1277-1284.
- 13
- 14 Bramley-Alves J, King DH, Robinson SA, Miller RE (2014) Dominating the Antarctic
15 environment: Bryophytes in a time of change. In: *Photosynthesis in Bryophytes and Early*
16 *Land Plants* (eds Hanson DT and Rice SK), PP. 309-324. Springer, Netherlands.
- 17
- 18 Bronk Ramsey C (1995) Radiocarbon calibration and analysis of stratigraphy: the OxCal
19 program. *Radiocarbon*, **37**, 425-430.
- 20
- 21 Bronk Ramsey C (2001) Development of the radiocarbon program. *Radiocarbon*, **43**, 355–
22 363.
- 23
- 24 Brugnoli E, Hubick KT, von Caemmerer S, Wong SC, Farquhar GD (1988) Correlation
25 between the carbon isotope discrimination in leaf starch and sugars of C_3 plants and the ratio

1 of intercellular and atmospheric partial pressures of carbon dioxide. *Plant Physiology*, **88**,
2 1418-1424.

3
4 Callaghan T, Sonesson M, Somme L (1992) Response of terrestrial plants and invertebrates to
5 environmental change at high latitudes. *Philosophical Transactions of the Royal Society of*
6 *London Series B*, **388**, 279-288.

7
8 Charman DJ, Barber KE, Blaauw M *et al.* (2009) Climate drivers for peatland palaeoclimate
9 records. *Quaternary Science Reviews*, **28**, 1811-1819.

10
11 Clarke LJ, Robinson SA, Hua Q, Ayre DJ, Fink D (2012) Radiocarbon bomb spike reveals
12 biological effects of Antarctic climate change. *Global Change Biology*, **18**, 301-310.

13
14 Convey P, Chown SL, Clarke A *et al.* (2014) The spatial structure of Antarctic biodiversity.
15 *Ecological Monographs*, **84**, 203-244.

16
17 Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant
18 ecology. *Annual Review of Ecology and Systematics*, **33**, 507-559.

19
20 Farquhar G, O'Leary M, Berry J (1982) On the relationship between carbon isotope
21 discrimination and the intercellular carbon dioxide concentration in leaves. *Functional Plant*
22 *Biology*, **9**, 121-137.

23
24 Farquhar GD, Ehleringer I, Hubick K (1989) Carbon isotope discrimination and
25 photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503-37.

- 1
- 2 Francey RJ, Allison CE, Etheridge DM *et al.* (1999) A 1000-year high precision record of
- 3 $\delta^{13}\text{C}$ in atmospheric CO_2 . *Tellus B*, **51**, 170-193.
- 4
- 5 Galimov EM (2000) Carbon isotope composition of Antarctic plants. *Geochimica et*
- 6 *Cosmochimica Acta*, **64**, 1737-1739.
- 7
- 8 Hietz P, Wanek W, Dnisch O (2005) Long-term trends in cellulose $\delta^{13}\text{C}$ and water-use
- 9 efficiency of tropical *Cedrela* and *Swietenia* from Brazil. *Tree Physiology*, **25**, 745-752.
- 10
- 11 Hua Q, Jacobsen GE, Zoppi U, Lawson EM, Williams AA, Smith AM, McGann MJ (2001)
- 12 Progress in radiocarbon target preparation at the ANTARES AMS centre. *Radiocarbon*, **43**,
- 13 275-282.
- 14
- 15 Hultine KR, Marshall JD (2000) Altitude trends in conifer leaf morphology and stable carbon
- 16 isotope composition. *Oecologia*, **123**, 32-40.
- 17
- 18 IPCC (2014) Freshwater Resources. In: *Climate change 2014: impacts, adaptation, and*
- 19 *vulnerability* (eds Kabat P and Kundzewicz Z), PP. 76. IPCC, Geneva.
- 20
- 21 Kaislahti Tillman PK, Holzkamper S, Kuhry P, Sannel ABK, Loader NJ, Robertson I (2010)
- 22 Long-term climate variability in continental subarctic Canada: A 6200-year record derived
- 23 from stable isotopes in peat. *Palaeogeography, Palaeoclimatology, Palaeoecology*, **298**, 235-
- 24 246.
- 25

- 1 Lajtha K, Getz J (1993) Photosynthesis and water use efficiency in *Pinyon-juniper*
2 communities along an elevation gradient in northern New Mexico. *Oecologia*, **94**, 95–111.
3
- 4 Lebouvier M, Laparie M, Hullé M *et al.* (2011) The significance of the sub-Antarctic
5 Kerguelen Islands for the assessment of the vulnerability of native communities to climate
6 change, alien insect invasions and plant viruses. *Biological Invasions*, **13**, 1195-1208.
7
- 8 Loader NJ, McCarroll D, Van der Knaap O, Robertson I, Gagen M (2007) Characterizing
9 carbon isotopic variability in *Sphagnum*. *The Holocene*, **17**, 403-410.
10
- 11 Lucieer A, Turner D, King DH, Robinson SA (2014) Using an Unmanned Aerial Vehicle
12 (UAV) to capture micro-topography of Antarctic moss beds. *International Journal of Applied*
13 *Earth Observation and Geoinformation*, **27, Part A**, 53-62.
14
- 15 Matsumoto GI, Tani Y, Seto K *et al.* (2011) Holocene paleolimnological changes in Lake
16 Skallen Oike in the Syowa Station area of Antarctica inferred from organic components in a
17 sediment core (Sk4C-02). *Journal of Paleolimnology*, **44**, 677-693.
18
- 19 Mayewski PA, Meredith MP, Summerhayes CP *et al.* (2009) State of the Antarctic and
20 Southern Ocean climate system. *Reviews of Geophysics*, **47**, 1003.
21
- 22 McCarroll D, Loader NJ (2004) Stable isotopes in tree rings. *Quaternary Science Reviews*,
23 **23**, 771-801.
24

1 Menot G, Burns SJ (2001) Carbon isotopes in ombrogenic peat bog plants as climatic
2 indicators: Calibration from an altitudinal transect in Switzerland. *Organic Geochemistry*, **32**,
3 233-245.

4
5 Menot-Combes G, Combes P-P, Burns SJ (2004) Climatic information from $\delta^{13}\text{C}$ in plants by
6 combining statistical and mechanistic approaches. *The Holocene*, **14**, 931-939.

7
8 Moschen R, Kihl N, Rehberger I, Licke A (2009) Stable carbon and oxygen isotopes in sub-
9 fossil *Sphagnum*: Assessment of their applicability for palaeoclimatology. *Chemical Geology*,
10 **259**, 262-272.

11
12 O'Leary MH (1981) Carbon isotope fractionation in plants. *Phytochemistry*, **20**, 553-567.

13
14 Pancost RD, Sinninghe Damste JS, Baas M, Geel B (2003) Response of an ombrotrophic bog
15 to a regional climate event revealed by macrofossil, molecular and carbon isotope data. *The*
16 *Holocene*, **13**, 921.

17
18 Rice SK (2000) Variation in carbon isotope discrimination within and among *Sphagnum*
19 species in a temperate wetland. *Oecologia*, **123**, 1-8.

20
21 Rice SK, Giles L (1996) The influence of water content and leaf anatomy on carbon isotope
22 discrimination and photosynthesis in *Sphagnum*. *Plant, Cell & Environment*, **19**, 118-124.

- 1 Rice SK, Schuepp PH (1995) On the ecological and evolutionary significance of branch and
2 leaf morphology in aquatic *Sphagnum* (Sphagnaceae). *American Journal of Botany*, **82**, 833-
3 846.
- 4
- 5 Roads E, Longton RE, Convey P (2014) Millennial timescale regeneration in a moss from
6 Antarctica. *Current Biology*, **24**. doi: <http://dx.doi.org/10.1016/j.cub.2014.01.053>.
- 7
- 8 Robinson SA, Erickson DJ (2014) Not just about sunburn – the ozone hole’s profound effect
9 on climate has significant implications for Southern Hemisphere ecosystems. *Global Change*
10 *Biology*. doi: 10.1111/gcb.12739.
- 11
- 12 Robinson SA, Osmond CB, Giles L (1993) Interpretations of gradients in $\delta^{13}\text{C}$ value in thick
13 photosynthetic tissues of plants with Crassulacean acid metabolism. *Planta*, **190**, 271-276.
- 14
- 15 Robinson SA, Wasley J, Tobin AK (2003) Living on the edge – plants and global change in
16 continental and maritime Antarctica. *Global Change Biology*, **9**, 1681-1717.
- 17
- 18 Royles J (2012) Environmental isotopic records preserved in Antarctic peat moss banks. PhD
19 Thesis, University of Cambridge, Cambridge.
- 20
- 21 Royles J, Amesbury MJ, Convey P, Griffiths H, Hodgson DA, Leng MJ, Charman DJ (2013)
22 Plants and soil microbes respond to recent warming on the Antarctic Peninsula. *Current*
23 *Biology*, **23**, 1702-1706.
- 24

1 Royles J, Griffiths H (2014) Climate change impacts in polar-regions: lessons from Antarctic
2 moss bank archives. *Global Change Biology*. doi: 10.1111/gcb.12774.

3
4 Royles J, Horwath AB, Griffiths H (2014) Interpreting bryophyte stable carbon isotope
5 composition: Plants as temporal and spatial climate recorders. *Geochemistry, Geophysics,*
6 *Geosystems*, **15**, 1462-1475.

7
8 Royles J, Ogée J, Wingate L, Hodgson DA, Convey P, Griffiths H (2012) Carbon isotope
9 evidence for recent climate-related enhancement of CO₂ assimilation and peat accumulation
10 rates in Antarctica. *Global Change Biology*, **18**, 3112-3124.

11
12 Rubino M, Etheridge DM, Trudinger CM *et al.* (2013) A revised 1000 year atmospheric $\delta^{13}\text{C}$ -
13 CO₂ record from Law Dome and South Pole, Antarctica. *Journal of Geophysical Research:*
14 *Atmospheres*, **118**, 8482-8499.

15
16 Sah S, Brumme R (2003) Altitudinal gradients of natural abundance of stable isotopes of
17 nitrogen and carbon in the needles and soil of a pine forest in Nepal. *Journal of Forest*
18 *Science*, **49**, 19-26.

19
20 Sawstrom C, Pearce I, Davidson AT, Rosen P, Laybourn-Parry J (2008) Influence of
21 environmental conditions, bacterial activity and viability on the viral component in 10
22 Antarctic lakes. *FEMS Microbiol Ecology*, **63**, 12-22.

23
24 Schleser GH, Frielingsdorf J, Blair A (1999) Carbon isotope behaviour in wood and cellulose
25 during artificial aging. *Chemical Geology*, **158**, 121-130.

1
2 Skrzypek G, Kaluzny A, Jedrysek MO (2007) Carbon stable isotope analyses of mosses: A
3 comparisons of bulk organic matter and extracted nitrocellulose. *Journal of the American*
4 *Society for Mass Spectrometry*, **18**, 1453-1458.

5
6 Sobek S, Tranvik J, Prairie Y, Kortelainen P, Cole J (2007) Patterns and regulation of
7 dissolved organic carbon: An analysis of 7,500 widely distributed lakes. *Limnology and*
8 *Oceanography*, **52**, 1208-1219.

9
10 Stanton DE, Merlin M, Bryant G, Ball MC (2014) Water redistribution determines
11 photosynthetic responses to warming and drying in two polar mosses. *Functional Plant*
12 *Biology*, **41**, 178-186.

13
14 Turner J, King J, Lachlan-Cope T, Jones P (2002) Climate change (Communication arising):
15 Recent temperature trends in the Antarctic. *Nature*, **418**, 291-292.

16
17 van Ommen T, Morgan V (2010) Snowfall increase in coastal East Antarctica linked with
18 southwest Western Australian drought. *Nature Geoscience*, **3**, 267-272.

19
20 Waite M, Sack L (2010) How does moss photosynthesis relate to leaf and canopy structure?
21 Trait relationships for 10 Hawaiian species of contrasting light habitats. *New Phytologist*, **185**,
22 156-72.

1 Wanek W, Heintel S, Richter A (2001) Preparation of starch and other carbon fractions from
2 higher plant leaves for stable carbon isotope analysis. *Rapid Communications in Mass*
3 *Spectrometry*, **15**, 1136–1140.

4
5 Wasley J, Robinson S, Lovelock C, Popp M (2006) Some like it wet – an endemic Antarctic
6 bryophyte likely to be threatened under climate change induced drying. *Functional Plant*
7 *Biology*, **33**, 443-55.

8
9 Wasley J, Robinson SA, Turnbull JD, King DH, Wanek W, Popp M (2012) Bryophyte
10 species composition over moisture gradients in the Windmill Islands, East Antarctica:
11 Development of a baseline for monitoring climate change impacts. *Biodiversity*, **13** 257-264.

12
13 Weng J, Chapple C (2010) The origin and evolution of lignin biosynthesis. *New Phytologist*,
14 **187**, 273-285.

15
16 Williams TG, Flanagan LB (1996) Effect of changes in water content on photosynthesis,
17 transpiration and discrimination against $\delta^{13}\text{C}$ in *Sphagnum*. *Oecologia*, **108**, 38-46.

18
19 Yamamoto S, Kawamura K, Seki O, Meyers PA, Zheng Y, Zhou W (2010)
20 Paleoenvironmental significance of compound-specific $\delta^{13}\text{C}$ variations in n-alkanes in the
21 Hongyuan peat sequence from southwest China over the last 13ka. *Organic Geochemistry*,
22 **41**, 491-497.

23
24 Zhang J, Marshall J, Jaquish B (1993) Genetic differentiation in carbon isotope discrimination
25 and gas exchange in *Pseudotsuga menziesii*. *Oecologia*, **93**, 80–87.

Supporting Information Legends

Fig. S1 Comparison between $\delta^{13}\text{C}_{\text{BULK}}$ and $\delta^{13}\text{C}_{\text{CELLULOSE}}$ in *Ceratodon purpureus*. Paired $\delta^{13}\text{C}_{\text{BULK}}$ - $\delta^{13}\text{C}_{\text{CELLULOSE}}$ samples were highly correlated ($y = 1.48x + 13.18$, $F_{1,8} = 33.1$, $df = 8$, $p < 0.0001$, $R^2 = 0.98$).

Fig. S2 Cell wall thickness in the Antarctic moss species, *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* collected from wet and dry environments on Robinson Ridge and Bailey peninsula in the Windmill Islands. Different letters indicate significant differences within and between species at $p = 0.05$. Crosses represent mean, lines represent median, boxes represent interquartile range, whiskers represent SD, $n = 50$. Magnification 40x.

Fig. S3 Mean (a) stem length, (b) length of photosynthetic section, (c) number of leaves and (d) leaf width of the Antarctic moss species *Bryum pseudotriquetrum*, grown in a temperature/light-controlled chamber for 5 weeks under 0, 25, 50, 75 and 100% cover, shown in (e). Different letters indicate significant differences between treatments at $p = 0.05$ using a Tukeys HSD test following significant ANOVA analyses. Error bars represent SD, $n = 5$.

Fig. S4 $\delta^{13}\text{C}_{\text{SUGAR}}$ difference between initial control plants and transplants after five weeks growth of *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* in the field in Antarctica. Mosses were transplanted from dry to dry (D-D), dry to intermediate (D-I), dry to wet (D-W), wet to dry (W-D), wet to intermediate (W-I) and wet to wet (W-W) conditions. Crosses represent mean, lines represent median, boxes represent interquartile range, whiskers represent SD, $n = 3$.

1 Tables

2 **Table 1** Isotopic change between wet and dry environments ($WD\delta^{13}C$) in sugar and cellulose compounds of
 3 three Antarctic moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* grown
 4 for 5 and 22 weeks.

Time	Analysis	Species $WD\delta^{13}C$		
		<i>C. purpureus</i>	<i>S. antarctici</i>	<i>B. pseudotriquetrum</i>
5 weeks	Sugar	1.35	1.03	1.64
	Cellulose	-0.91	0.74	1.73
22 weeks	Sugar	3.0	0.45	2.0
	Cellulose	2.9	0.5	1.8

1 **Table 2** $\delta^{13}\text{C}_{\text{CELLULOSE}}$ in three Antarctic moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and
2 *Schistidium antarctici*, kept in a chamber for 5 weeks under wet, intermediate or dry conditions. Data are means
3 (standard error), n = 6 - 8.

Environment	Species		
	<i>C. purpureus</i>	<i>S. antarctici</i>	<i>B. pseudotriquetrum</i>
Dry	-20.8 (0.5)	-20 (0.5)	-21.3 (0.5)
Intermediate	-19.7 (0.5)	-18.3 (0.5)	-19.7 (0.5)
Wet	-21.7 (0.5)	-19.3 (0.5)	-19.5 (0.5)

Figures

Fig. 1 Map of Antarctica showing the location of Automatic Weather Stations (AWS) where weather observation data is collected (as of 2009). Note poor coverage especially around the coast of East Antarctica. In addition most of these AWS were installed recently (1990 onwards). Insert shows the location of the three study sites: S1 Clark Peninsula, S2 Bailey Peninsula and S3 Robinson Ridge outlined in red. Adapted from University of Wisconsin - Madison (Space Science & Engineering Center, 2012) and Australian Antarctic Division Data Centre (Australian Antarctic Division, 2012). N.B. inset is inverted relative to main map orientation.

Fig. 2 $\delta^{13}\text{C}_{\text{BULK}}$ in the Antarctic moss species, *Schistidium antarctici*, *Ceratodon purpureus* and *Bryum pseudotriquetrum*, collected from locations assessed as representing dry, intermediate and wet environments on Robinson Ridge, and Clark and Bailey peninsulas (see Fig 1 for details). Different letters indicate significant differences at $p = 0.05$ between environments. Crosses represent mean, lines represent median, boxes represent interquartile range, whiskers represent SD, $n = 20 - 30$.

Fig. 3 $\delta^{13}\text{C}_{\text{CELLULOSE}}$ of *Ceratodon purpureus* moss sampled from wet, intermediate and dry environments and correlated with spot estimates of turf water content (TWC, g H₂O g⁻¹ dwt) at each sampling point. Samples were collected within Antarctic Specially Protected Area 135, on Bailey Peninsula and Robinson Ridge (see Fig 1) at sites previously identified as part of a permanent long-term monitoring study (Robinson, 2009). Prior estimations of long-term water environment are indicated by open circles for dry environments, crosses for intermediate environments and closed circles for wet environments. The relationship between $\delta^{13}\text{C}$ and TWC in *C. purpureus* was significant ($n = 22$, $y = 0.6x - 25.1$; $p < 0.0007$, $R^2 = 0.44$).

Fig. 4 $\delta^{13}\text{C}_{\text{SUGAR}}$ in three Antarctic moss species *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici*. Mosses were grown in a controlled growth chamber for 5 weeks under wet, intermediate or dry conditions (see text for details). Different letters indicate significant differences between environments at $p = 0.05$. Crosses represent mean, lines represent median, boxes represent interquartile range, whiskers represent SD, $n = 6 - 8$.

Fig. 5 $\delta^{13}\text{C}_{\text{SUGAR}}$ (a, b) and $\delta^{13}\text{C}_{\text{CELLULOSE}}$ (c, d) in three Antarctic moss species grown under either dry or wet conditions (a, c) for 22 weeks. Species variation for *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* is also shown (b, d). Mosses were either fully submerged (wet) or left exposed to the air and watered sparingly from below (dry). Different letters indicate significant differences between species and water treatments at $p = 0.05$. Crosses represent mean, lines represent median, boxes represent interquartile range, whiskers represent SD, $n = (\text{a-c}) 21$ and $(\text{b-d}) 16$.

Fig. 6 Comparisons between $\delta^{13}\text{C}_{\text{CELLULOSE}}$ and $\delta^{13}\text{C}_{\text{SUGAR}}$ in split samples of moss grown for
 (a) 5 weeks; *Bryum pseudotriquetrum*, *Ceratodon purpureus* and *Schistidium antarctici* and
 (b) 22 weeks; *B. pseudotriquetrum* (BP, dotted line, $n = 13$, $y = 1.14x - 4.85$; $R^2 = 0.69$, $P = 0.001$), *C. purpureus* (CP, solid line, $n = 13$, $y = 0.85x - 2.7$; $R^2 = 0.41$, $P = 0.01$) and *S. antarctici* (SA, dashed line, $n = 13$, $y = 0.81x - 3.73$; $R^2 = 0.57$, $P = 0.001$).

Fig. 7 Trends in $\delta^{13}\text{C}_{\text{GRAPHITE}}$ down three Antarctic moss cores of *Ceratodon purpureus* collected from Clark and Bailey peninsulas (see Fig 1 for details), remodeled from Clarke *et al.*, (2012). Cores from Whitney Point (green line, $n = 11$, $y = -125.14 + 0.05x - 0.002*(x - 1974.17)^2 - 0.0001*(x - 1974.17)^3$; $R^2 = 0.67$, $P = 0.02$), Red Shed (Red line, $n = 10$, $y = -0.04x + 62.80$; $R^2 = 0.65$, $P = 0.002$) and Antarctic Specially Protected Area 135 (ASPA, pink line, $n = 9$, $y = -0.03x + 42.84$; $R^2 = 0.56$, $P = 0.01$) are assessed against calculated $\delta^{13}\text{C}$ and TWC ($\text{g H}_2\text{O g}^{-1} \text{dwt}$) ranges for current Windmill Islands wet (blue), intermediate (white) and dry (yellow) environments.