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Moss δ13C: implications for subantarctic palaeohydrological reconstructions

Jessica Bramley-Alves
University of Wollongong, jba605@uowmail.edu.au

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

Sharon A. Robinson
University of Wollongong, sharonr@uow.edu.au

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Moss $\delta^{13}C$: Implications for subantarctic palaeohydrological reconstructions

Jessica Bramley-Alves[1,3], Wolfgang Wanek[2] and Sharon A. Robinson[1]

[1]Centre for Ecosystem Solutions, University of Wollongong, New South Wales 2522 Australia.

[2]Faculty of Life Sciences, Department of Microbiology and Ecosystem Science, University of Vienna, Austria.

Corresponding author: Jessica Bramley-Alves, T +65 9667 9327, jba605@gmail.com.[3]Curent address: Center for Urban Ecology and Greenery, National Parks Board, 1 Cluny Road, Singapore 259569.

1.0 Abstract

Southern Ocean Islands, despite their equitable oceanic climates, have recently experienced a number of pronounced climate variations. Shifts in water availability in this region are of concern; however, methods of measuring water availability are currently inadequate. Recent advances using stable carbon isotopes ($\delta^{13}C$) in Antarctic mosses to record long-term variations in water availability suggest that this technique might be applicable in other locations where conditions are cold enough to produce meaningful moss growth for reconstructions. Verification of this technique at each new location is essential, however, due to disparity between species and climates. Here, variations in $\delta^{13}C_{BULK}$ with growth water availability were measured in three moss species on subantarctic Macquarie Island. We found these subantarctic mosses showed no difference in $\delta^{13}C_{BULK}$ signatures between growth water environments and displayed more negative $\delta^{13}C_{BULK}$ ranges than those from East Antarctica, suggesting that climatic differences override the microclimate signal. Despite significant differences in leaf cell morphology there was no variation in $\delta^{13}C_{BULK}$ between these subantarctic species. It maybe that these species are unsuitable as biological proxies due to their growth form being less dense than the turf forming Antarctic species. This underlines the need to carryout preliminary research into moss carbon isotope fractionation for each new region, and for each species, where palaeohydrological reconstructions are planned – a step that is often not given appropriate consideration in palaeo-research.

Keywords: $\delta^{13}C$, subantarctic, cell wall thickness, climate change, proxies, bio-available water.

1.1 Introduction

The subantarctic region is seasonally one of the most stable regions of the world in terms of temperature and preciptiation, due chiefly to the influence of the Southern Ocean. Large variations in climate, however, have been reported over the past century across a range of Southern Ocean Islands (SOI; Fig.1a; Christensen et al., 2007; Pendlebury & Barnes-Keoghan, 2007; Turner et al., 2014). General trends show a rise in wind speed and temperature and a decrease in water availability and glaciation, with meteorological evidence presenting an increase in surface temperature on some SOI of greater than 0.6°C since the late 19th century (see Supporting Information Table S1 for temperature trends). Whilst data from pollen records show changes in climate have occurred in this region in the past (Scott 1985) it is the unique combination of warming and drying that makes current climate change so damaging to biota (Bergstrom et al., 2015; Le Roux and McGeoch 2008). Effects have already been identified on population range, size, nutrient demand, ecology and phenology for a number of subantarctic species including mammals (Budd, 2000; Louise Allan et al., 2013), plants (Bergstrom et al., 2015; Whinam and Copson, 2006; Frenot et al., 2001; Smith and Steenkamp 1990; Smith, 2002), invertebrates (Bokhorst et al., 2008; Hanel et al., 1998; Lebouvier et al., 2011; McGeoch et al., 2006; Slabber and Chown 2005) and birds (Budd, 2000; Delord et al., 2004; Weimerskirch et al., 2003).

Of particular concern are the effects that changes to bioaccessible water across the SOI will have, as even small fluctuations to bio-accessible water are likely to produce a large impact on subantarctic organisms which have evolved under stable conditions (Bergstrom et al., 2015; Bergstrom et al., 2006; Bergstrom & Chown, 1999; IPCC, 2014) and

consequently have low genetic variation (Taylor, 1995). Current observations and future predictions of water availability, however, rely on information collected from weather stations, most of which lack long-term records, and are spatially disparate. Moreover, weather stations often only measure one form of precipitation (rain) and fail to account for other forms (snow, mist, fog) that contribute equally to the water balance, leading to misinterpretation of the biological implications of change (Bramley-Alves et al., 2015; Ingraham & Mark, 2000). The use of carbon isotopes ($\delta^{13}C$) in subantarctic mosses to record long-term variations in water availability could represent a fine-scale, high resolution technique to fill in such observational gaps.

1.1.1 Stable carbon isotope fractionation in mosses and their use as a proxy for water availability

Stable isotopes continue to prove their immense applicability across a range of scientific disciplines (Eiler et al., 2014), especially as recorders of climate variation in plant material (Loisel et al., 2010; Loisel et al., 2009; Kaislahti Tillman et al., 2010b; Schleser et al., 1999). In plants, carbon dioxide - including its isotopically heavy and light forms, $^{13}CO_2$ and $^{12}CO_2$ – is assimilated by the carboxylating enzyme, ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO; Park and Epstein 1960). Carbon dioxide molecules containing the heavier isotope, $^{13}CO_2$, have lower kinetic velocities and stronger covalent bonds and therefore have slower reaction rates when compared to their $^{12}CO_2$ counterparts (Melander and Saunders 1980). Consequently, lower diffusional speeds of the heavier $^{13}CO_2$ molecules cause some degree of isotope fractionation. The majority of isotope fractionation, however, occurs inside the chloroplasts where the

enzyme RuBisCO selectively favours the incorporation of isotopically lighter $^{12}CO_2$ molecules. This results in the partitioning of $^{13}C/^{12}C$ isotopes (expressed in delta notation ($\delta^{13}C$)) between photosynthates and atmospheric CO_2, the plants being biased towards lighter ^{12}C isotopes (Farquhar et al. 1989, Brugnoli and Farquhar 2004).

Recent studies have demonstrated the suitability of carbon isotopes to accurately record long-term variations in water availability in intact shoots of moss in Antarctica (Bramley-Alves et al., 2015). Mosses, which are widely distributed in coastal regions of Antarctica and on the subantarctic islands, incorporate carbon isotope signals in a sequential fashion - similar to tree rings - providing the chronostratigraphic basis to interpret temporal trends in water availability when paired with precision dating via the ^{14}C 'bomb pulse' method (Clarke et al., 2012; Royles & Griffiths, 2015; Royles et al., 2013; Royles et al., 2012). Stable isotope signatures captured within each moss shoot segment have been found to represent the CO_2 diffusional restrictions as affected by moss water availability. The latter integrates the balance between water inputs (meltwater, snow) and outputs (evapotranspiration, runoff) from moss patches, and constitutes the immediate growth water environment of the moss (Bramley-Alves et al., 2015). The moss water environment is most importantly affected by changes in annual precipitation, growing season temperature, wind, relative humidity and insolation, and their interactions.

The ability of mosses to reflect bioavailable water in their $\delta^{13}C$ is a result of their simple physiology. Unlike vascular plants, mosses lack stomata meaning that they cannot regulate their carbon dioxide or water uptake through any physiological response, and therefore mosses in terms of water relations are poikilohydric (Rice, 2000; Williams &

Flanagan, 1996). Consequently, the resistance to CO_2 diffusion in moss is altered by the presence or absence of a water film on the moss surface, which can create large barriers to carbon assimilation due to the much lower CO_2 diffusivity in water than in air (Rice & Giles, 1996). $\delta^{13}C$ values are highest (less discrimination against isotopically heavier $^{13}CO_2$) when plants are covered by diffusion limiting water films resulting in a less negative $\delta^{13}C$ signature. Alternatively, $\delta^{13}C$ is more negative when plants are drier and diffusion and photosynthetic rates are maximised, as RuBisCO consistently favours the isotopically lighter $^{12}CO_2$ molecules (Rice, 2000). In the short Antarctic summer melt season the moss environment continually moves between these water extremes. The $\delta^{13}C$ value within each section of shoot represents the integration of all the carbon assimilation that has occurred under different conditions over a given timeframe. This technique therefore allows assessment of the water availability of a region – through $\delta^{13}C$ as a proxy measure – at a high temporal resolution (Bramley-Alves et al., 2015). The use of live non-vascular species for palaeoclimatic reconstructions in polar regions remains largely unexplored, due in part to the lack of long-term information found in bryophytes growing in temperate regions. While these short-lived bryophytes may be capable of providing readily interpreted climate information, they are of little use if they can only provide a few years of data. By contrast, polar mosses grow extremely slowly and therefore have the potential to provide centuries of past climate data in a single intact shoot. The success of $\delta^{13}C$ as a proxy for water availability in Antarctica advocates its use in other cold climate regions, such as the subantarctic, where growth rates are slow enough to produce meaningful information and where existing data regarding water

availability and its climatic drivers (e.g. changes in temperature, precipitation, wind, and insulation) is similarly limited.

1.1.2 Climatic considerations on carbon isotope fractionation in mosses

Abiotic factors, other than water availability, such as light intensity and temperature have also been shown to influence $\delta^{13}C$ in plants (Menot and Burns, 2001; Menot-Combes et al., 2004; Kaislahti Tillman et al., 2010a; Skrzypek et al., 2007b). Studies have demonstrated that temperature either directly influences chemical or physical process associated with ^{13}C isotope fractionation or indirectly alters plant metabolism through affecting conductance or assimilation rate (Schleser, 1995; Vogel et al., 1970). Laboratory and field studies seeking to quantify the effect of temperature on plant metabolism, surprisingly, contradict one another. Almost all moss field studies suggest an increase in $\delta^{13}C$ is associated with rising temperatures (Menot & Burns 2001; Menot-Combes et al., 2004) whereas the majority of laboratory studies propose opposite trends (O'Leary, 1988). The effect of light on $\delta^{13}C$ under controlled environment and field studies is similarly inconsistent. The physiological effect associated with an increase in light intensity, however, is usually an increase in assimilation rate within the plant and a subsequent $\delta^{13}C$ increase. According to those studies conducted under controlled conditions, there is approximately a 2‰ increase in $\delta^{13}C$ in vascular plants for a fivefold increase in light intensity (O'Leary, 1988).

Climatic conditions between the previously tested Antarctic environment (Bramley-Alves et al., 2015) and the subantarctic environment are categorically different (Fig. 2). Macquarie Island supports year round conditions which are warmer and wetter than

Casey Station in East Antarctica with less seasonal fluctuation (Fig. 2). Annual mean daily sunshine hours are 3.2 and 2.4 at Casey and Macquarie Island, respectively (http://www.bom.gov.au/). How these conditions affect $\delta^{13}C$ in subantarctic moss is not yet known making it imperative to verify the capacity of local subantarctic moss species to differentiate between wet and dry conditions through $\delta^{13}C$ before moss $\delta^{13}C$ can be implemented as a palaeohydrological proxy in this region.

1.1.3 Moss morphological effects on carbon isotope fractionation

Morphological differences between moss species have also been shown to significantly affect carbon isotope fractionation of moss by influencing the CO_2 diffusion resistance and subsequent carbon assimilation (Bramley-Alves et al., 2015; Menot & Burns, 2001; Rice & Schuepp, 1995; Stanton et al., 2014). Cell wall thickness and growth habit of Antarctic species, for example, were thought to cause significant species discrepancies in $\delta^{13}C$ (Bramley-Alves et al., 2015). Previous research demonstrated that plant growth form in particular may influence diffusional resistance of CO_2 into cells as it affects the development of surface water films or can alter the thickness of boundary layers surrounding plant surfaces (Rice & Schuepp, 1995). This highlights the need to first test carbon isotope fractionation at a species level and is supported by previous research showing significant species-specific $\delta^{13}C$ differences within similar environments (Brader et al., 2010; Loader et al., 2007; Menot-Combes et al., 2004; Moschen et al. 2009; Pancost et al, 2003; Zhang et al., 1993).

With these considerations in mind we seek to expand the use of $\delta^{13}C$ in moss as a proxy of growth water environment by examining the carbon isotope fractionation in three

subantarctic moss species collected from inundated (wet) and intermediate water environments as well as elevated (dry) locations. Specifically we examine: (1) the influence of growth water environment on $\delta^{13}C_{BULK}$ in subantarctic moss under field conditions; (2) inter-species variability; (3) differences and similarities in $\delta^{13}C_{BULK}$ in mosses between Antarctic and subantarctic locations; (4) differences between maximum air temperature and moss surface temperature between Antarctic and subantarctic locations; and (5) differences in morphological traits between species which may influence $\delta^{13}C_{BULK}$. The success of this method would facilitate the establishment of unstudied water availability trends and their drivers for the subantarctic region and support extending this proxy to other cold climate environments. If subantarctic mosses are found not to differentiate between growth water environments through $\delta^{13}C_{BULK}$, however, then this research will prevent prospective reconstructive studies in the subantarctic from erroneous interpretations in the future and foster the search for other proxies of water environment and climate change.

1.2 Methods

1.2.1 Subantarctic study site and species

Macquarie Island was selected as the subantarctic study location. The island, an emergent part of the Macquarie Ridge, lies in isolated waters midway between Tasmania, New Zealand and the Antarctic continent (54°38'S and 158°53'E; Fig. 1b). It was formed by crustal accretion (Christodoulou et al., 1984) resulting in a large undulating plateau, 34 km long by 5.5 km wide, that rises steeply from the narrow coastal fringe to a height

of 240-250 m above sea level. Vegetation is restricted to only 46 species of native vascular flora, with a complete absence of trees, but an abundance of grasses and other herb species relative to the number of vascular plant species (Seppelt 2004). Bryophyte species, *Breutelia pendula* (Sm.) Mitt, *Brachythecium austro-salebrosum* (C. Muell.) Kindb. and *Sanionia uncinata* (Hedwig), were selected for this study due to their identifiable features and abundant distribution across water gradients on the plateau.

1.2.2 Subantarctic sample collection

For each of the three study species, moss plugs (~ 2 cm^2 by 4 cm deep) were collected from areas in which moss was observed to grow in one of three different water environments. Water environments were classified based on an elevational/topographic gradient from a running stream. Moss growing in or directly beside a stream was classified as 'inundated', mid-way up the bank was classified as 'intermediate' and those growing higher up the bank, with no direct access to stream water were classified as 'elevated'. These environment classifications were deemed to be the most accurate mirror of the 'wet', 'intermediate' and 'dry' conditions of the comparable Antarctic study (Bramley-Alves et al., 2015). Once collected, samples were oven dried to a constant mass at 60°C and returned to Australia/Austria for analysis.

1.2.3 Sample preparation

Samples were prepared for $\delta^{13}C_{BULK}$ and $\delta^{13}C_{CELLULOSE}$ analysis as described in Bramley-Alves et al., (2015). Green growing tips (between 2 – 4 mm) were used, within which growth rates are expected to be similar between species. Carbon isotope analyses were

conducted at the University of Vienna with a continuous-flow Isotope 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 ratio of ^{13}C relative to ^{12}C in samples was calculated by the following equation:

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

where R is the atomic ratio of ^{13}C to ^{12}C. The $\delta^{13}C$ 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 materials (International Atomic Energy Agency, Vienna, Austria). Typical standard deviations of $\delta^{13}C$ analyses for replicate samples are ± 0.1‰.

As $\delta^{13}C_{CELLULOSE}$ values provided similar results to $\delta^{13}C_{BULK}$ we selected $\delta^{13}C_{BULK}$ values to conduct our analysis in order to allow comparison to past studies (Bramley-Alves et al., 2015; Skrzypek et al., 2007a; Table S2).

1.2.4 Comparisons of $\delta^{13}C_{BULK}$ in mosses between Antarctic and subantarctic locations

In our equivalent Antarctic investigation (Bramley-Alves et al., 2015), we explored the effect of water environment on the $\delta^{13}C_{BULK}$ of three Antarctic moss species (*Bryum pseudotriquetrum* (Hedw.) Gaertn., Meyer & Scherb., *Ceratodon purpureus* (Hedw.) Brid.and *Schistidium antarctici* Card.) across three different Antarctic locations. As the differences in moss $\delta^{13}C_{BULK}$ between wet-dry environments described by Bramley-Alves et al., (2015) were statistically similar between all study locations for all species, moss

$\delta^{13}C_{BULK}$ comparisons between Antarctic and Macquarie Island species were assessed using a single Antarctic location (Bailey peninsula) to ensure similar sampling sizes for statistical analysis.

1.2.5 Moss surface temperature regimes at Antarctic and subantarctic locations

The difference between maximum air temperature and moss surface temperature over diurnal time scales was assessed at both locations using iButtons fixed to the surface of the moss, or cushioned within sponge, which has been found to be a reliable substitute for moss turf (King, 2009). Antarctic moss surface temperatures over the period of active growth were captured using three iButtons (December 2012 – January 2013). Subantarctic moss surface temperatures were captured using 12 iButtons over the months of April and May 2015, as growth activity occurs year round. Other climatic parameters over these periods were retrieved from the Australian Bureau of Meteorology.

1.2.6 Variations in morphological features between Antarctic and subantarctic species

Cell wall thickness of subantarctic moss species was examined in samples collected from intermediate water environments as described in Bramley-Alves et al., (2015). Results were then compared to those of Antarctic species collected from intermediate environments in Bramley-Alves et al., (2015). Growth habit of all species was examined.

1.2.7 Statistical analysis

The change in $\delta^{13}C_{BULK}$ between environments (wet-dry difference: $\delta^{13}C_{W\text{-}D}$) was calculated by subtracting the mean dry $\delta^{13}C_{BULK}$ from the mean wet $\delta^{13}C_{BULK}$. 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. Differences in $\delta^{13}C_{BULK}$ between subantarctic environments and species were assessed using a two-way ANOVA, with $\delta^{13}C_{BULK}$ as the dependent factor and environment and species as the main effects alongside their interaction term. Differences in cell wall thickness and in $\delta^{13}C_{BULK}$ between moss species from Antarctic and subantarctic sites were examined using a one-way ANOVA, with species as the main effect. Where significant interactions were found Tukey HSD *post hoc* tests were used to identify significantly different means.

1.3 Results

1.3.1 The influence of growth water environment and species on $\delta^{13}C_{BULK}$ under subantarctic field conditions

There was no significant interaction between selected moss species and water environment on Macquarie Island ($F_{53,\ 45} = 0.79$, $p = 0.53$). $\delta^{13}C_{BULK}$ did not differ between subantarctic species ($F_{53,\ 45} = 0.36$, $p = 0.70$), with all species showing a similar range of $\delta^{13}C_{BULK}$ values (Fig. 3). Moreover, moss $\delta^{13}C_{BULK}$ did not differ significantly between growth water environments on Macquarie Island ($F_{53,\ 45} = 1.91$, $p = 0.16$). However, while not significant, a trend was apparent with mean $\delta^{13}C_{BULK}$ values in wet sites less negative (-26.6 ± 0.4‰) than either intermediate (-27.4 ± 0.4‰) or dry sites (-

27.9 ± 0.4‰), with a $\delta^{13}C_{W\text{-}D}$ of 1.3‰ (Fig. 3). This trend was particularly clear in *B. pendula*.

1.3.2 Comparisons of $\delta^{13}C_{BULK}$ in mosses between Antarctic and subantarctic locations

Subantarctic moss species, *B. pendula*, *B. austro-salebrosum* and *S. uncinata,* collected from Macquarie Island had significantly more negative $\delta^{13}C_{BULK}$ signatures than the Antarctic species *S. antarctici* and *C. purpureus* and less positive, but statistically similar, $\delta^{13}C_{BULK}$ signatures when compared to the Antarctic species *B. pseudotriquetrum* ($F_{104, 109} = 12.67$, $p = 0.001$, Fig. 4).

1.3.3 Variations in morphological features between Antarctic and subantarctic species

Differences between cell wall thickness were statistically significant among all three subantarctic species ($F_{2, 2} = 84.44$, $p = 0.0001$; Fig. 5) with the mean width of cell walls in *B. pendula* 1.5 and 1.8 times greater than *B. austro-salebrosum* and *S. uncinata*, respectively.

The cell wall thickness of subantarctic moss species fell within the range of cell wall thicknesses of Antarctic moss species (Table 1). Antarctic moss species displayed both the thinnest and the thickest cell walls (*Bryum pseudotriquetrum* and *Schistidium antarctici*, respectively) of all six moss species, with subantarctic values intermediate between the two.

The growth habit of two of the three Antarctic species - *C.purpureus* and *S. antarctici* - were deemed to be of a compact fashion, forming tightly bound turves (Bramley-Alves et

al., 2015). The third Antarctic species *B.pseudotriquetrum* and all three subantarctic species displayed a loose weft growth habit (Table 1).

1.3.4 Moss surface temperature regimes between Antarctic and subantarctic locations

Antarctic moss surface temperature, during a period of active growth, was more variable under Antarctic conditions than subantarctic conditions (Fig. 6). Moss temperature at Casey station displayed consistent diurnal temperature cycling, dipping below zero overnight and up above 15°C during the day. This is evident by the large standard deviation associated with the mean moss temperature at this site (4.2°C ± 6.8). The moss surface temperature at Macquarie Island showed less fluctuation and, with the exception of a brief and very unusual freezing period around the 12th of April 2015, maintained its temperature above 15 °C, with an average temperature of 17.1°C ± 5.3.

1.4 Discussion

Recent work demonstrated the accuracy of $\delta^{13}C$ in Antarctic moss as a proxy for long-term variation in water availability (Bramley-Alves et al., 2015). Here we examined if this technique could be extended to the subantarctic region by exploring $\delta^{13}C$ variation in mosses on subantarctic Macquarie Island. We reveal that, unlike in the Antarctic environment, the moss species growing under subantarctic conditions did not show significant differences in $\delta^{13}C_{BULK}$ between wet and dry environments in the field. This suggests that reconstructions of past water environments using moss carbon isotope analysis may not be applicable under subantarctic environments. Possible reasons for this include the subantarctic climate and the study species growth form.

While the absence of significant changes in $\delta^{13}C_{BULK}$ between wet, intermediate and dry subantarctic sites is "unfortunate" from a proxy point of view, it was not entirely unexpected. Carbon isotope values in mosses are influenced by two extreme environmental conditions: when the leaf surface is dry and diffusion and photosynthetic rates are maximized thus favouring the uptake of lighter $^{12}CO_2$ (Rice, 2000) or, in contrast, when the surface is covered by a water film which creates a strong barrier to CO_2 diffusion and carbon assimilation resulting in less carbon isotope fractionation and increased assimilation of heavier $^{13}CO_2$ (Rice & Giles, 1996). We suggest that the higher prevalence of water in the subantarctic environment (in the form of fog, mist, rain and sleet) triggers the continual presence of thin (< 2 mm) water films on the surface of moss that masks potential differences between wet and dry sites. Climatically, Antarctica and the subantarctic are very different (Fig. 2). The Antarctic air is very dry, and while it is colder than the subantarctic, solar radiation can heat up the surface of moss beds to over 25°C within the course of a day (Fig. 6a) - giving rise to large evaporative loss on clear sunny days (Bramley-Alves et al., 2014). As a result, moisture from intermittent precipitation inputs, such as falling snow, dries up rapidly allowing for a clear dry $\delta^{13}C_{BULK}$ signal to establish in areas of moss beds that are not submerged or covered by thick water films (Bramley-Alves, unpublished data). In contrast Macquarie Island supports consistent cloud cover, high relative humidity and slight, yet measurable, precipitation in the form of misty rain and fog on an average of 313 days per year (Bureau of Meteorology, 2015). The plateau, where mosses are most abundant, is often shrouded with mist or fog for days at a time. Not only does this make the selection of 'wet' and 'dry' sites challenging, but it probably also means that there are very few

occasions where the leaf surface of moss is completely dry. As a result there are likely only limited occasions where a dry (or truly wet) $\delta^{13}C_{BULK}$ signal can properly establish. On the continent wet sites are around melt lakes or along melt streams, providing frequent inundation and are probably almost continuously wet throughout the short summer melt season (4 – 6 weeks).

The high variability in $\delta^{13}C_{BULK}$ observed in the Macquarie Island moss species also suggest that these subantarctic locations maybe more variable in their water environments over time or across sites on the island than those at Casey Station in East Antarctica. Growth of moss probably occurs throughout the year with larger seasonal changes in water availability possibly masking the water signature, especially if mosses grow most under each species optimum water availability. The short season on the continent means that mosses probably acclimate to the dryness of their site (Robinson et al., 2000) and have to maximize growth during summer melt since growth over the rest of the year is impossible. These results emphasise the importance of testing carbon isotope fractionation in each prospective environment before carrying out palaeohydrological reconstructions and contributes to evidence that carbon isotope fractionation in bryophytes changes across environmental gradients and climates (Hultine & Marshall, 2000; Lajtha & Getz, 1993; Menot-Combes et al., 2004; Sah & Brumme, 2003). It is important to note that, although not significant, moss $\delta^{13}C_{BULK}$ in this study was trending in the expected direction, with mosses from wet sites showing less negative $\delta^{13}C_{BULK}$ than dry sites and a $\delta^{13}C_{W\text{-}D}$ equal to 1.3‰. This was particularly evident in the species *B. pendula* (Fig. 3). In previous Antarctic studies a $\delta^{13}C_{W\text{-}D}$ equal to 1.6‰ produced significant differences between water environments (Bramley-Alves et al., 2015)

suggesting that future investigations in the subantarctic region, possibly across multiple islands, with greater replication and more accurate microclimate and hydrological measurements supplemented by moss water relation analysis could potentially uncover significant $\delta^{13}C_{BULK}$ differences between these environments. If sites are more similar within single subantarctic islands, studies of the same species across multiple islands might provide sufficient range in water environments to reveal a clear trend.

Not only did we find no difference in $\delta^{13}C_{BULK}$ between wet, intermediate and dry locations on Macquarie Island but we also found no difference within the three subantarctic moss species. This contrasts to previous findings in Antarctica where the three moss species showed significantly different $\delta^{13}C_{BULK}$ signatures under field conditions (Bramley-Alves et al., 2015), and to the results of other studies, which suggest that polar mosses show a high degree of inter-specific carbon isotope differences (Galimov, 2000; Lee et al., 2009). It is possible, however, that the similarities seen in $\delta^{13}C_{BULK}$ between subantarctic moss species are also a result of the homogeneity of the subantarctic environment. Whilst $\delta^{13}C_{BULK}$ differed between Antarctic species in the field, species signatures become less distinct when grown for prolonged periods under homogenous chamber conditions (Bramley-Alves et al., 2015). The constancy of the subantarctic climatic conditions may mask any potential isotopic differences between species. For example, the propensity for similar water films to cover moss on a day-to-day basis whenever it is foggy in the subantarctic environment suggests that the diffusional resistance maybe alike in all species regardless of morphological differences in cell wall thickness, such as those observed here (Fig. 5).

Interestingly, while the $\delta^{13}C_{BULK}$ signal of subantarctic mosses was within the range of other moss species in the region (Supporting Information, Table S2) it was more negative than the $\delta^{13}C_{BULK}$ signal of moss species from East Antarctica (Fig. 6). We had predicted that the continually moist environmental conditions in the subantarctic would lead to greater diffusional limitation and thereby cause a decline in carbon isotope fractionation and ultimately more positive $\delta^{13}C_{BULK}$ signals. This discrepancy for subantarctic species could be caused by either physiological differences between Antarctic and subantarctic moss species or difference in abiotic factors that cause divergence of moss $\delta^{13}C_{BULK}$ from expectations. At the physiological level we would expect leaf morphology or plant growth form to influence $\delta^{13}C_{BULK}$ as found in Bramley-Alves et al., (2015). Comparisons of cell wall widths between the two polar locations, however, show that the range of all three subantarctic species was within that of the Antarctic species (Table 1), ruling this out as a possible reason for a more positive $\delta^{13}C_{BULK}$ in Antarctic species. Growth form, on the other hand, is distinctly different between the Antarctic species *S. antarctici* and *C. purpureus* when compared to the Antarctic species *B. pseudotriquetrum* and all three of the subantarctic species from Macquarie Island (Table 1). Both *S. antarctici* and *C. purpureus* grow in compact turfs or cushions, which often undergo prolonged periods of submersion and have a strong tendency to retain thick water films on their turfs. Conversely, subantarctic species and the Antarctic species *B. pseudotriquetrum* do not grow in such a compact fashion but rather as loose wefts, and their photosynthetically active tips are consequently less likely to be completely submerged or covered by thick water films. These species are expected to be covered by thin films of water condensed on to the growing leaf surface from the atmosphere which

rapidly equilibrates with cell contents and the atmosphere. This would likely lead to less diffusional resistance and more negative $\delta^{13}C_{BULK}$. Extended periods of complete submergence of Antarctic species *S. antarctici* and *C. purpureus* during the summer melt could lead to a more positive $\delta^{13}C_{BULK}$ than for subantarctic moss. The statistically similar $\delta^{13}C_{BULK}$ signatures between the subantarctic mosses and Antarctic *B. pseudotriquetrum* support growth habit as a potential influence on $\delta^{13}C_{BULK}$. Possibly these looser growth forms are therefore less suitable as proxies for water than those species that form a compact turf. Chamber studies would be a useful way to establish if these weft-like species develop clearly differentiated carbon isotope signatures under controlled wet and dry conditions, as shown for Antarctic turf mosses. As shown in Bramley-Alves et al., (2015) such experiments allow mosses to be maintained submerged or moist, and enable the full extent of the dry to wet range in $\delta^{13}C_{BULK}$, to be observed.

A number of abiotic factors, such as light, temperature, nutrients and CO_2 partial pressure have been shown to influence moss $\delta^{13}C$ (Kaislahti Tillman et al., 2010a; Menot & Burns, 2001; Menot-Combes et al., 2004; Skrzypek et al., 2007b). Temperature is obviously very different between the Antarctic and the subantarctic environments, with mean subantarctic summer air temperatures ~8°C higher than Antarctic air temperatures during the Antarctic growing season (Fig. 2). By observing the moss surface temperature of Antarctic and subantarctic moss beds during periods of active growth we found that moss surface temperature in Antarctica is diurnally highly variable, whereas the mean moss surface temperature on Macquarie Island is consistently higher and temperatures far more stable than seen in Antarctica despite being recorded two months later in the year (Fig. 6 a & b). Subantarctic turf temperature remained close to the mosses likely optimal

temperature of approximately 15 - 20°C over the study period (Davey and Rothery 1996; Collins 1977; Ashcroft et al., in review), with the exception of a rare freezing event. Antarctic moss turfs, on the other hand, experience sub zero temperatures almost daily, with day time temperatures rising above 15°C for a few hours around midday. Studies suggest that higher moss surface temperatures may cause CO_2 diffusion through water to increase less than RuBisCO activity and consequently trigger greater diffusional limitation of photosynthesis leading to less carbon isotope fractionation (O'Leary, 1988). Therefore we would expect the higher moss surface temperatures in the subantarctic to produce less carbon isotope fractionation than in the comparatively colder Antarctic environment, and thus more positive $\delta^{13}C_{BULK}$ signatures, however this was not the case. Other field studies suggest that an increase in temperature is linked to a decrease in $\delta^{13}C$ (Menot-Combes et al., 2004; Skrzypek et al., 2007b). For example, a study examining the effect of temperature changes along altitudinal transects on $\delta^{13}C$ in two moss species, *Sphagnum* and *Polytrichum*, found a clear -1.6‰ and -1.5‰ decrease in $\delta^{13}C$ corresponding to a 1°C increase in air temperature, respectively (Skrzypek et al., 2007b). This shift in isotope fractionation in relation to temperature is very large and if we were to apply it to the difference in temperature between Antarctica and the subantarctic we would project almost a 12‰ difference which is unrealistic. Another study examining *Sphagnum* species in peat bogs sampled across an altitudinal transect linked shifts of between -0.4 and -0.2‰ in $\delta^{13}C$ to a 1°C increase in temperature (Menot & Burns, 2001), however, further investigations by the same authors concluded the inverse trend: that $\delta^{13}C$ becomes more positive in *Sphagnum* at higher temperatures (Menot-Combes et al., 2004). Our current knowledge of moss $\delta^{13}C$ values suggest that $\delta^{13}C$ shows a negative,

but weak, trend with increasing temperature across Antarctic and subantarctic locations (Supporting Information, Fig. S1a). Similarly moss species $\delta^{13}C$ show a negative, but weak, trend with increasing annual rainfall (Supporting Information, Fig. S1b). Clearly this is an area that warrants further investigation with a single species, perhaps the widly distributed species *S. uncinata*, across multiple locations.

We suggest that the influence of growth form and temperature on $\delta^{13}C_{BULK}$, alongside other environmental factors, such as light and nutrients, should be examined across a number of subantarctic and maritime antarctic islands. Lower ambient light intensity during growth on Macquarie Island, for example, may cause lower carbon assimilation rates (lower RuBisCO) but similar CO_2 diffusion rates in mosses (Farquhar et al., 1989; O'Leary 1981). This would suggest increased carbon isotope fractionation in the subantarctic and may add to the temperature and growth form effect. Larger sampling sizes and more advanced measures to estimate microsite water availability may also uncover differences in $\delta^{13}C_{BULK}$ between growth water environments on subantarctic or maritime Antarctic islands on which precipitation is more variable than on Macquarie Island.

1.5 Conclusions

The use of $\delta^{13}C_{BULK}$ in subantarctic mosses as a proxy for growth water environment is complicated by a range of climatic factors that make temporal environmental interpretation challenging. These findings currently limit the application of moss $\delta^{13}C$ as a climate proxy in the subantarctic on the scale of single islands. However, it is possible that a larger sampling size, a wider range of test species and more accurate definitions of

what constitutes wet and dry sites may elucidate isotopic shifts between growth water environments across subantarctic locations. Additionally further study of the impact of turf morphology on carbon isotope fractionation in mosses would establish if certain growth forms are more suitable as biological proxies. Importantly, this study provides baseline information for future studies exploring this method in the subantarctic and reiterates the need to test carbon isotope fractionation in target species under field conditions, as well as under controlled conditions, before proxy methods using moss $\delta^{13}C$ as an indicator of growth water environment can be relied upon.

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References

Ashcroft, M.B., Casanova-Katny, A., Mengersen, K., Rosenstiel, T.N., Turnbull, J.D., Wasley, J., Waterman, M.J., Zuniga, G.E. & Robinson, S.A. (in review). Bayesian methods for comparing species physiological and ecological response curves. *Ecological Informatics* ECOINF-D-15-00149.

Bergstrom, D.M., Bricher, P., Raymond, B., Terauds, A., Doley, D., McGeoche, M.A., Whinam, J., Glen, M., Yuan, Z., Kiefer, K., Shaw, J.D., Bramley-Alves, J., Rudman, T., Mohammed, C., Lucieer, A., Visoiu, M., Jansen van Vuuren, B., Ball, M.C., 2015. Rapid collapse of a sub-Antarctic alpine ecosystem. Journal of Applied Ecology 52, 774-783.

Bergstrom, D.M., Convey, P., Huiskes, A.H.L., 2006. Antarctic climate change and its influences on terrestrial ecosystems. In: Trends in Antarctic Terrestrial and Limnetic Ecosystems, 253-272. Springer Netherlands.

Bergstrom, D.M., Chown, S., 1999. Life at the front: history, ecology and change on southern ocean islands. TREE 14, 472-477.

Bokhorst, S., Huiskes, A., Convey, P., van Bodegom, P.M., Aerts, R., 2008. Climate change effects on soil arthropod communities from the Falkland Islands and the Maritime Antarctic. Soil Biology and Biochemistry 40, 1547-1556.

Brader, A.V., van Winden, J.F., Bohncke, S.J.P., Beets, C.J., Reichert, G., de Leeuw, J., 2010. Fractionation of hydrogen, oxygen and carbon isotopes in n-alkanes and cellulose of three *Sphagnum* species. Organic Geochemistry 41, 1277-1284.

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

Bramley-Alves J., King D.H., Robinson S.A., Miller R.E., 2014. Dominating the Antarctic environment: Bryophytes in a time of change. In: Photosynthesis in Bryophytes and Early Land Plants (eds. Hanson, D.T. and Rice S.K.), PP. 309-324. Springer, Netherlands.

Brugnoli E. and Farquhar G. (2004). Photosynthetic fractionation of carbon isotopes. *Photosynthesis*. Leegood R., Sharkey T. and Caemmerer S., Springer Netherlands. **9:** 399-434.

Budd, G.M., 2000. Changes in Heard Island glaciers, king penguins and fur seals since 1947. In: Heard Island Papers (eds. Banks M. and Brown M.), PP. 47-60. Papers and Proceedings of the Royal Society of Tasmania, Hobart.

Bureau of Meteorology, 2015. http://shop.bom.gov.au/. Accessed 24/11/2015.

Christensen, J., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R., Kwon, W., Laprise, R., Magaña Rueda, V., Mearns, L., Menéndez, C., Räisänen, J., Rinke, A., Sarr, A., Whetton, P., 2007. Regional climate projections. Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change United Kingdom and New York, NY, USA, Cambridge University Press.

51 Christodoulou, C., Griffin, R., Foden, I., 1984. The geology of Macquarie Island. ANARE
52 Research Notes 21, 1-15.
53
54 Clarke, L.J., Robinson, S.A., Hua, Q., Ayre, D.J., Fink, D., 2012. Radiocarbon bomb spike
55 reveals biological effects of Antarctic climate change. Global Change Biology 18, 301-310.
56
57 Delord, K., Barbraud, C., Weimerskirch, H., 2004. Recent changes in the population size of
58 king penguins: environmental variability or density dependence? Polar Biology 27, 793-800.
59
60 Eiler, J.M., Bergquist, B., Bourg, I., Cartigny, P., Farquhar, J., Gagnon, A., Guo, W., Halevy,
61 I., Hofmann, A., Larson, T.E., Levin, N., Schauble, E.A., Stolper, D., 2014. Frontiers of
62 stable isotope geoscience. Chemical Geology 372, 119-113.
63
64 Farquhar, G.D., Ehleringer, I., Hubick, K., 1989. Carbon isotope discrimination and
65 photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 40, 503-37.
66
67 Frenot, Y., Gloaguen, J., Masse´, L., Lebouvier, M., 2001. Human activities, ecosystem
68 disturbance and plant invasions in subantarctic Crozet, Kerguelen and Amsterdam Islands.
69 Biological Conservation 101, 33–50.
70
71 Galimov, E.M., 2000. Carbon isotope composition of Antarctic plants. Geochimica et
72 Cosmochimica Acta 64, 1737-1739.
73
74 Hanel, C., Chown, S., Davies, L., 1998. Records of alien insect species from sub-Antarctic
75 Marion and South Georgia Islands. African Entomology 6, 366-369.

Hultine, K.R., Marshall, J.D., 2000. Altitude trends in conifer leaf morphology and stable carbon isotope composition. Oecologia 123, 32-40.

Ingraham, N.L., Mark, A.F., 2000. Isotopic assessment of the hydrologic importance of fog deposition on tall snow tussock grass on southern New Zealand uplands. Austral Ecology 25, 402-408.

IPCC (2014) Freshwater Resources. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability (eds Kabat P and Kundzewicz Z) PP. 76. IPCC, Geneva.

Jones, A.G., Chown, S.L., Ryan, P.G., Gremmen, N.J.M., Gaston, K.J., 2003. A review of conservation threats on Gough Island: a case study for terrestrial conservation in the Southern Oceans. Biological Conservation, 113, 75-87.

Kaislahti Tillman, P.K., Holzkmper, S., Kuhry, P., Sannel, A., Britta, K., Loader, N.J., Robertson, I., 2010a. Stable carbon and oxygen isotopes in *Sphagnum fuscum* peat from subarctic Canada: Implications for palaeoclimate studies. Chemical Geology 270, 216-226.

Kaislahti Tillman, P.K., Holzkamper, S., Kuhry, P., Sannel, A.B.K., Loader, N.J., Robertson, I. 2010b. Long-term climate variability in continental subarctic Canada: A 6200-year record derived from stable isotopes in peat. Palaeogeography Palaeoclimatology Palaeoecology 298, 235-246.

King, D., 2009. Rapid change in continental Antarctic vegetation communities. BEnvSci

Honours dissertation, University of Wollongong.

Lajtha, K., Getz, J., 1993. Photosynthesis and water use efficiency in *Pinyon-juniper* communities along an elevation gradient in northern New Mexico. Oecologia 94, 95–111.

Le Roux, P.C., McGeoch, M.A., 2008. Rapid range expansion and community reorganization in response to warming. Global Change Biology 14, 2950-2962.

Lebouvier, M., Laparie, M., Hullé, M., Marais, A., Cozic, Y., Lalouette, L., Vernon, P., Candresse, T., Frenot, Y., Renault, D., 2011. The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses. Biological Invasions 13, 1195-1208.

Lee, Y., Lim, H., Yoon, H., 2009. Carbon and nitrogen isotope composition of vegetation on King George Island, maritime Antarctic. Polar Biology 32, 1607-1615.

Loader, N.J., McCarroll, D., Van der Knaap, O., Robertson, I., Gagen, M., 2007. Characterizing carbon isotopic variability in *Sphagnum*. The Holocene 17, 403-410.

Loisel, J., Garneau, M., Helie, J., 2010. *Sphagnum* $\delta^{13}C$ values as indicators of palaeohydrological changes in a peat bog. The Holocene 20, 285-291.

Loisel, J., Garneau, M., Hélie, J.F., 2009. Modern *Sphagnum* $\delta^{13}C$ signatures follow a surface moisture gradient in two boreal peat bogs, James Bay lowlands, Québec. Journal of Quaternary Science 24, 209-214.

126

127 Louise Allan, E., William Froneman, P., Durgadoo, J.V., McQuaid, C.D., Ansorge, I.J.,
128 Richoux, N.B., 2013. Critical indirect effects of climate change on sub-Antarctic ecosystem
129 functioning. Ecology and Evolution 3, 2994-3004.

130

131 McGeoch, M.A., Le Roux, P.C., Hugo, E.A., Chown, S.L., 2006. Species and community
132 responses to short-term climate manipulation: Microarthropods in the sub-Antarctic. Austral
133 Ecology 31, 719-731.

134

135 Melander L. and Saunders W. H. (1980). Reaction Rates of Isotopic Molecules. New York,
136 Wiley.

137

138 Menot, G., Burns, S.J., 2001. Carbon isotopes in ombrogenic peat bog plants as climatic
139 indicators: Calibration from an altitudinal transect in Switzerland. Organic Geochemistry 32,
140 233-245.

141

142 Menot-Combes, G., Combes, P.P., Burns, S.J., 2004. Climatic information from $\delta^{13}C$ in
143 plants by combining statistical and mechanistic approaches. The Holocene14, 931-939.

144

145 Moschen, R., Kihl, N., Rehberger, I., Licke, A., 2009. Stable carbon and oxygen isotopes in
146 sub-fossil *Sphagnum*: Assessment of their applicability for palaeoclimatology. Chemical
147 Geology 259, 262-272.

148

149 O'Leary, M.H., 1981. Carbon isotope fractionation in plants. Phytochemistry 20, 553-567.

150

O'Leary, M.H., 1988. Carbon isotopes in photosynthesis. BioScience 38, 328-336.

Pancost, R.D., Sinninghe Damste, J.S., Baas, M., Geel, B., 2003. Response of an ombrotrophic bog to a regional climate event revealed by macrofossil, molecular and carbon isotope data. The Holocene 13, 921.

Park R. and Epstein S. (1960). Carbon isotope fractionation during photosynthesis. *Geochimica et Cosmochimica Acta* **21**(1,12): 110-126.

Pendlebury, S.F., Barnes-Keoghan, I.P., 2007. Climate and climate change in the sub-Antarctic. Papers and Proceedings of the Royal Society of Tasmania 141, 67-82.

Rice, S.K., 2000. Variation in carbon isotope discrimination within and among *Sphagnum* species in a temperate wetland. Oecologia 123, 1-8.

Rice, S.K., Giles, L., 1996. The influence of water content and leaf anatomy on carbon isotope discrimination and photosynthesis in *Sphagnum*. Plant, Cell & Environment 19, 118-124.

Rice, S.K., Schuepp, P.H., 1995. On the ecological and evolutionary significance of branch and leaf morphology in aquatic *Sphagnum* (Sphagnaceae). American Journal of Botany 82, 833- 846.

Robinson, S.A., Wasley, J., Popp, M., Lovelock, C.E., 2000. Desiccation tolerance of three moss species from continental Antarctica. Functional Plant Biology 27, 379-388.

Royles, J., Griffiths, H., 2015. Invited review: climate change impacts in polar regions: lessons from Antarctic moss bank archives. Global Change Biology, 21, 1041–1057.

Royles, J., Amesbury, M.J., Convey, P., Griffiths, H., Hodgson, D.A., Leng, M.J., Charman, D.J., 2013. Plants and soil microbes respond to recent warming on the Antarctic Peninsula. Current Biology 23, 1702-1706.

Royles, J., Ogée, J., Wingate, L., Hodgson, D.A., Convey, P., Griffiths, H., 2012. Carbon isotope evidence for recent climate-related enhancement of CO_2 assimilation and peat accumulation rates in Antarctica. Global Change Biology 18, 3112-3124.

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

Schleser, G.H., Helle, G., Lücke, A., Vos, H., 1999. Isotope signals as climate proxies: the role of transfer functions in the study of terrestrial archives. Quaternary Science Reviews 18, 927-943.

Schleser, G.H., 1995. Parameters determining carbon isotope ratios in plants. In: Frenzel, B. (Ed.), Problems of stable isotopes in tree-rings, lake sediments and peat-bogs as climatic evidence for the Holocene. Gustav Fischer, München 15, 71–96.

200 Scott, L., 1985. Palynological Indications of the Quaternary Vegetation History of Marion
201 Island (Sub-Antarctic). Journal of Biogeography 12, 413-431.
202
203 Skrzypek, G., Kaluzny, A., Jedrysek, M.O., 2007a. Carbon stable isotope analyses of mosses:
204 A comparisons of bulk organic matter and extracted nitrocellulose. Journal of the American
205 Society for Mass Spectrometry 18, 1453-1458.
206
207 Skrzypek, G., Kaluzny, A., Wojtun, B., Jedrysek, M.O., 2007b. The carbon stable isotopic
208 composition of mosses: A record of temperature variation. Organic Geochemistry 38, 1770-
209 1781.
210
211 Slabber, S., Chown, S.L., 2005. Differential responses of thermal tolerance to acclimation in
212 the sub-Antarctic rove beetle Halmaeusa atriceps. Physiological Entomology 30, 195-204.
213
214 Smith, V.R., 2002. Climate Change in the Sub-Antarctic: An Illustration from Marion Island.
215 Climatic Change 52, 345-357.
216
217 Smith, V., Steenkamp, M., 1990. Climate change and its ecological implications at a
218 subantarctic island. Oecologia 85, 14-24.
219
220 Stanton, D.E., Merlin, M., Bryant, G., Ball, M.C., 2014. Water redistribution determines
221 photosynthetic responses to warming and drying in two polar mosses. Functional Plant
222 Biology 41, 178-186.
223
224 Taylor, B.W., 1995. The flora, vegetation and soils of Macquarie Island. ANARE Reports 2.

225

226 Turner, J., Barrand, N., Bracegurdle, T., Convey, P., Hodgson, D.A., Jarvis, M., Jenkins, A.,
227 Marshall, G., Meredith, M.P., Roscoe, H., Shanklin, J., Fench, J., Goosse, H., Guglielmin,
228 M., Gutt, J., Jacobs, S., Kennicutt II, M.C., Masson-Delmotte, V., Mayewski, P.A., Nararro,
229 F., Robinson, S.A., Scambos, T.A., Sparrow, M., Summerhayes, C., Speer, K., Klepikov, A.,
230 2014. Antarctic climate change and the environment: an update. Polar Record 50, 237-259.

231

232 Weimerskirch, H., Inchausti, P., Guinet, C., 2003. Trends in birds and seals populations as
233 indicators of a system shift in the Southern Ocean. Antarctic Science 15, 249-256.

234

235 Whinam, J., Copson, G., 2006. *Sphagnum* moss: an indicator of climate change in the sub-
236 Antarctic. Polar Record 42, 43-49

237

238 Williams, T.G., Flanagan, L.B., 1996. Effect of changes in water content on photosynthesis,
239 transpiration and discrimination against $\delta^{13}C$ in *Sphagnum*. Oecologia 108, 38-46.

240

241 Zhang, J., Marshall, J., Jaquish, B., 1993. Genetic differentiation in carbon isotope
242 discrimination and gas exchange in *Pseudotsuga menziesii*. Oecologia 93, 80–87.

Supporting Information

Table S1

Summary of recent temperature changes on a range of Southern Ocean Islands that have exhibited the most pronounced changes. Temp = temperature.

	Island						
	Kerguelen	**Heard**	**Marion**	**Gough**	**Macquarie**	**Amsterdam**	**Campbell**
Temperature trend	Significant rise in mean temp of 1.3°C between 1960s and the1990s. Furthermore, annual temps were warmer and more stable between 1976 and 2008 (4.84 ± 0.27°C) than over the period 1951 to 1975 (4.30 ± 0.44°C).	Rise in mean temp of 0.8°C since the late 1940s.	Significant mean temp increase between the 1950s - 1990s with a 1.2°C increase between 1967 and 1999. Furthermore, mean temp of five hottest years in the same period increased from 5.5 to 6.8°C. Daily maxima and minima increased by 0.28°C and 0.24°C (respectively) per decade.	Rise in mean temp of 0.6°C between 1963 and the year 2000.	A 0.3°C increase in the mean surface temp between 1912 and 1948 with a total warming of 0.6°C between 1912 and 1998. Current temps are 1°C higher than in 1949.	Cooling during the 1960s, followed by an increase between the start of the 1970s and the end of the 1980s. Since then, mean temps have remained approximately 1°C higher than in the 1960s.	An increase of 0.6°C in mean summer temps and 0.4°C in mean winter temps between 1971 and 2003. It is estimated that temps have warmed by 0.5°C and 1°C since 1895.
References	Frenot et al., (1997); Smith (2002); Lebouvier et al., (2011)	BOM (2015)	Smith (2002); Le Roux and McGeoch (2008)	Jones et al., (2003)	Tweedie and Bergstrom (2000); Adamson et al., (1988); Jacka et al., (2004)	Weimerskirch et al., (2003)	Mc Gone et al., (2007)

Table S2

Bulk carbon isotope ($\delta^{13}C$) ranges and calculated means of moss species from subantarctic (SOI) and maritime Antarctic locations. Data are from the current study and the literature. (*) indicates $\delta^{13}C$ value based on a single measurement, other $\delta^{13}C$ values are based on 2+measurements.

Species	Location	Bulk range $\delta^{13}C$ (‰)	Mean $\delta^{13}C$ (‰)	Reference
Andreaea depressinervis	Signy Island (SOI)	-23.87*	-23.87*	Galimov 2000
Andreaea depressinervis	Aitcho Island (SOI)	-23.6*	-23.6*	Galimov 2000
Andreaea regularis	King George Island, maritime Antarctica	-25.4 to -22.9	-24.2 ± 0.9	Lee et al., 2009
Batramia patens	King George Island, maritime Antarctica	-28*	-28*	Lee et al., 2009
Brachythecium austro-salebrosum	Macquarie Island (SOI)	-30.1 to -24.0	-27.5 ± 1.8	Current study
Breutelia pendula	Macquarie Island (SOI)	-29.2 to -22.4	-27.0 ± 1.8	Current study
Bryum pseudotriquetrum	King George Island, maritime Antarctica	-25.6 to -24.7	-25.1± 0.3	Lee et al., 2009
Bryum pseudotriquetrum	Windmill Island, East Antarctica	-27.8 to -22.0	-26.1± 1.2	Bramley-Alves et al., 2015
Bryum sp.	Signy Island (SOI)	-22.47*	-22.47*	Galimov 2000
Ceratodon purpureus	Windmill Island, East Antarctica	-28.4 to -20.4	-25.1± 1.4	Bramley-Alves et al., 2015
Chorisodontium aciphyllum	Signy Island (SOI)	-24.8 to -23.4	-23.7 ± 0.3	Galimov 2000
Chorisodontium aciphyllum	Signy Island (SOI)	-24.0 to -20.5	NA	Royles 2012
Chorisodontium aciphyllum	King George Island, maritime Antarctica	-24.7*	-24.7*	Lee et al., 2009
Drepanocladus sp.	Greenwich Island (SOI)	-24.86 to -23.49	-24.1± 0.48	Galimov 2000
Drepanocladus sp.	Aitcho Island (SOI)	-24.43*	-24.43*	Galimov 2000
Drepanocladus uncinatus	Signy Island (SOI)	-23.86*	-23.86*	Galimov 2000
Drepanocladus uncinatus	Signy Island (SOI)	-24.12*	-24.12*	Galimov 2000

Drepanocladus uncinatus	Gibbs Island (SOI)	-24.29*	-24.29*	Galimov 2000
Polytrichum alpestra	Signy Island (SOI)	-25.21*	-25.21*	Galimov 2000
Polytrichum alpinum	Signy Island (SOI)	-25.86*	-25.86*	Galimov 2000
Polytrichastrum alpinum	King George Island, maritime Antarctica	-27.3*	-27.3*	Lee et al., 2009
Polytrichastrum strictum	King George Island, maritime Antarctica	-28.2 to -26	-26.9± 0.8	Lee et al., 2009
Sanionia georgico-uncinata	King George Island, maritime Antarctica	-27.9 to -24	-26.1 ± 1.5	Lee et al., 2009
Sanionia uncinata	Macquarie Island (SOI)	-30.3 to -24	-27.5 ± 2.1	Current study
Schistidium antarctici	Windmill Island, East Antarctica	-28.3 to 20.9	-24.5 ± 1.4	Bramley-Alves et al., 2015

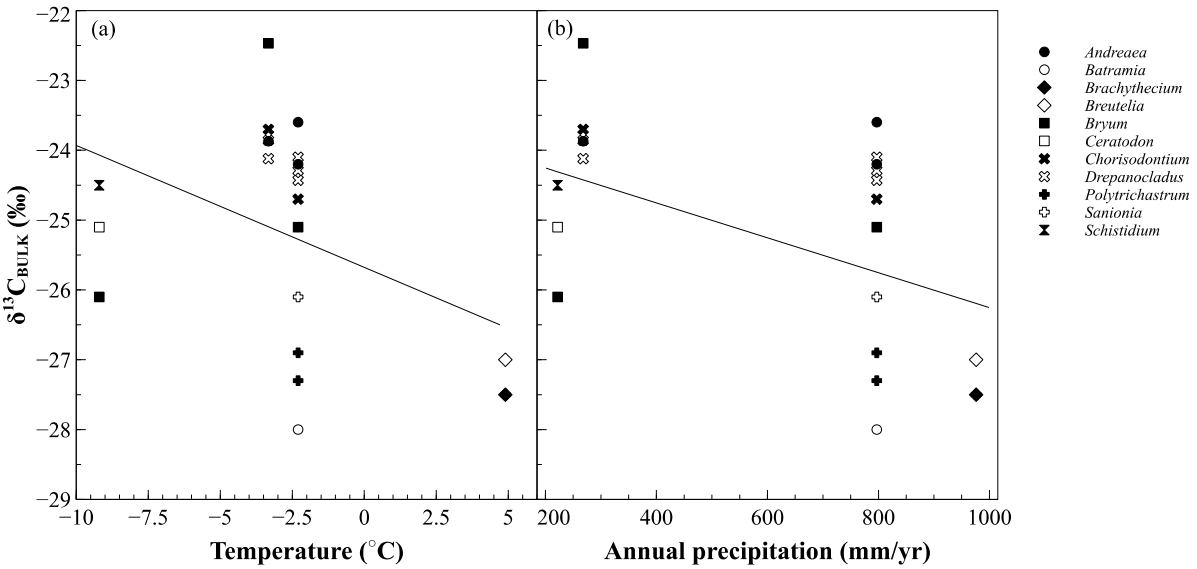

Fig. S1. The relationship between (a) mean annual temperature (y = -1.0x - 28.14, R^2 = 0.17) and (b) annual precipitation (y = -97.36x – 1863.2, R^2 = 0.24), when compared to the mean $\delta^{13}C_{BULK}$ of moss growing in subantarctic and maritime Antarctic locations. Data are from the current study and the literature.

Supporting Information References

Adamson, D., Whetton, P., Selkirk, P., 1988. An analysis of air temperature records for Macquarie Island: decadal warming, ENSO cooling and Southern Hemisphere circulation patterns. Papers and Proceedings Royal Society of Tasmania 122, 107–112.

Bureau of Meteorology, 2015. http://shop.bom.gov.au/. Accessed 24/11/2015.

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

Frenot, Y., Gloaguen, J., Tre´hen, P., 1997. Climate change in Kerguelen Islands and colonization of recently deglaciated areas by *Poa kerguelensis* and *P. annua*. Antarctic Communities: Species, Structure and Survival. Battaglia, B., Valencia, J., Walton, D., (Eds). Cambridge, Cambridge University Press**,** 358– 366.

Galimov, E.M., 2000. Carbon isotope composition of Antarctic plants. Geochimica et Cosmochimica Acta 64, 1737-1739.

Jacka, T., Budd, W., Holder, A., 2004. A further assessment of surface temperature changes at stations in the Antarctic and Southern Ocean, 1949–2002. *Annals of Glaciology* 39, 331–338.

Jones, A.G., Chown, S.L., Ryan, P.G., Gremmen, N.J.M., Gaston, K.J., 2003. A review of conservation threats on Gough Island: a case study for terrestrial conservation in the Southern Oceans. Biological Conservation, 113, 75-87.

Lebouvier, M., Laparie, M., Hullé, M., Marais, A., Cozic, Y., Lalouette, L., Vernon, P., Candresse, T., Frenot, Y., Renault, D., 2011. The significance of the sub-Antarctic Kerguelen Islands for the assessment of the vulnerability of native communities to climate change, alien insect invasions and plant viruses. Biological Invasions 13, 1195-1208.

Lee, Y., Lim, H., Yoon, H., 2009. Carbon and nitrogen isotope composition of vegetation on King George Island, maritime Antarctic. Polar Biology 32, 1607-1615.

Le Roux, P.C., McGeoch, M.A., 2008. Rapid range expansion and community reorganization in response to warming. Global Change Biology 14, 2950-2962.

Mc Gone, M., Wilmshurst, J., Meurk, J., 2007. Climate, fire farming and the recent vegetation history of subantarctic Campbell Island. Earth and Environmental Science Transactions of the Royal Society of Edinburgh 98, 71-84.

Royles J., 2012. Environmental isotopic records preserved in Antarctic peat moss banks. Doctor of Philosophy, University of Cambridge.

Smith, V.R., 2002. Climate Change in the Sub-Antarctic: An Illustration from Marion Island. Climatic Change 52, 345-357.

Tweedie, C., Bergstrom, D., 2000. A climate change scenario for surface air temperature at sub Antarctic Macquarie Island. In: Davison, W., Howard-Williams, C., and Broady, P.A. (eds) Antarctic Ecosystems: Models for Wider Understanding. Christchurch, New Zealand Natural Sciences, Christchurch, New Zealand, pp. 272-281.

Weimerskirch, H., Inchausti, P., Guinet, C., 2003. Trends in birds and seals populations as indicators of a system shift in the Southern Ocean. Antarctic Science 15, 249-256.