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

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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 ($\delta^{13}\text{C}$) 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 $\delta^{13}\text{C}_{\text{BULK}}$ with growth water availability were measured in three moss species on subantarctic Macquarie Island. We found these subantarctic mosses showed no difference in $\delta^{13}\text{C}_{\text{BULK}}$ signatures between growth water environments and displayed more negative $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ between these subantarctic species. It may be 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 carry out 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.

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

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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 ($\delta^{13}\text{C}$) 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 $\delta^{13}\text{C}_{\text{BULK}}$ with growth water availability were measured in three moss species on subantarctic Macquarie Island. We found these subantarctic mosses showed no difference in $\delta^{13}\text{C}_{\text{BULK}}$ signatures between growth water environments and displayed more negative $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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: $\delta^{13}\text{C}$, 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 precipitation, 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 ($\delta^{13}\text{C}$) 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, $^{13}\text{CO}_2$ and $^{12}\text{CO}_2$ - is assimilated by the carboxylating enzyme, ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO; Park and Epstein 1960). Carbon dioxide molecules containing the heavier isotope, $^{13}\text{CO}_2$, have lower kinetic velocities and stronger covalent bonds and therefore have slower reaction rates when compared to their $^{12}\text{CO}_2$ counterparts (Melander and Saunders 1980). Consequently, lower diffusional speeds of the heavier $^{13}\text{CO}_2$ 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 $^{12}\text{CO}_2$ molecules. This results in the partitioning of $^{13}\text{C}/^{12}\text{C}$ isotopes (expressed in delta notation ($\delta^{13}\text{C}$)) 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 $\delta^{13}\text{C}$ 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 CO₂ 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 CO₂ diffusivity in water than in air (Rice & Giles, 1996). $\delta^{13}\text{C}$ values are highest (less discrimination against isotopically heavier ¹³CO₂) when plants are covered by diffusion limiting water films resulting in a less negative $\delta^{13}\text{C}$ signature. Alternatively, $\delta^{13}\text{C}$ is more negative when plants are drier and diffusion and photosynthetic rates are maximised, as RuBisCO consistently favours the isotopically lighter ¹²CO₂ molecules (Rice, 2000). In the short Antarctic summer melt season the moss environment continually moves between these water extremes. The $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ increase. According to those studies conducted under controlled conditions, there is approximately a 2‰ increase in $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ before moss $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ (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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ in subantarctic moss under field conditions; (2) inter-species variability; (3) differences and similarities in $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$. 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 $\delta^{13}\text{C}_{\text{BULK}}$, 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 ($\sim 2 \text{ cm}^2$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ and $\delta^{13}\text{C}_{\text{CELLULOSE}}$ 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 ¹³C relative to ¹²C 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 atomic ratio of ¹³C to ¹²C. 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 CO₂ reference gas, calibrated to IAEA-CH-6 and IAEA-CH-7 reference materials (International Atomic Energy Agency, Vienna, Austria). Typical standard deviations of $\delta^{13}\text{C}$ analyses for replicate samples are $\pm 0.1\text{‰}$.

As $\delta^{13}\text{C}_{\text{CELLULOSE}}$ values provided similar results to $\delta^{13}\text{C}_{\text{BULK}}$ we selected $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ between wet-dry environments described by Bramley-Alves et al., (2015) were statistically similar between all study locations for all species, moss

$\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ between environments (wet-dry difference: $\delta^{13}\text{C}_{\text{W-D}}$) was calculated by subtracting the mean dry $\delta^{13}\text{C}_{\text{BULK}}$ from the mean wet $\delta^{13}\text{C}_{\text{BULK}}$. 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 $\delta^{13}\text{C}_{\text{BULK}}$ between subantarctic environments and species were assessed using a two-way ANOVA, with $\delta^{13}\text{C}_{\text{BULK}}$ as the dependent factor and environment and species as the main effects alongside their interaction term. Differences in cell wall thickness and in $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ under subantarctic field conditions

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

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

1.3.2 Comparisons of $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ signatures than the Antarctic species *S. antarctici* and *C. purpureus* and less positive, but statistically similar, $\delta^{13}\text{C}_{\text{BULK}}$ 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 ($4.2^{\circ}\text{C} \pm 6.8$). 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 $17.1^{\circ}\text{C} \pm 5.3$.

1.4 Discussion

Recent work demonstrated the accuracy of $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $^{12}\text{CO}_2$ (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 $^{13}\text{CO}_2$ (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 $\delta^{13}\text{C}_{\text{BULK}}$ 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) $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ in this study was trending in the expected direction, with mosses from wet sites showing less negative $\delta^{13}\text{C}_{\text{BULK}}$ than dry sites and a $\delta^{13}\text{C}_{\text{W-D}}$ equal to 1.3‰. This was particularly evident in the species *B. pendula* (Fig. 3). In previous Antarctic studies a $\delta^{13}\text{C}_{\text{W-D}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ between subantarctic moss species are also a result of the homogeneity of the subantarctic environment. Whilst $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ from expectations. At the physiological level we would expect leaf morphology or plant growth form to influence $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$. Extended periods of complete submergence of Antarctic species *S. antarctici* and *C. purpureus* during the summer melt could lead to a more positive $\delta^{13}\text{C}_{\text{BULK}}$ than for subantarctic moss. The statistically similar $\delta^{13}\text{C}_{\text{BULK}}$ signatures between the subantarctic mosses and Antarctic *B. pseudotriquetrum* support growth habit as a potential influence on $\delta^{13}\text{C}_{\text{BULK}}$. 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 $\delta^{13}\text{C}_{\text{BULK}}$, to be observed.

A number of abiotic factors, such as light, temperature, nutrients and CO_2 partial pressure have been shown to influence moss $\delta^{13}\text{C}$ (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 $\sim 8^\circ\text{C}$ 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 CO₂ 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 $\delta^{13}\text{C}_{\text{BULK}}$ signatures, however this was not the case. Other field studies suggest that an increase in temperature is linked to a decrease in $\delta^{13}\text{C}$ (Menot-Combes et al., 2004; Skrzypek et al., 2007b). For example, a study examining the effect of temperature changes along altitudinal transects on $\delta^{13}\text{C}$ in two moss species, *Sphagnum* and *Polytrichum*, found a clear -1.6‰ and -1.5‰ decrease in $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ to a 1°C increase in temperature (Menot & Burns, 2001), however, further investigations by the same authors concluded the inverse trend: that $\delta^{13}\text{C}$ becomes more positive in *Sphagnum* at higher temperatures (Menot-Combes et al., 2004). Our current knowledge of moss $\delta^{13}\text{C}$ values suggest that $\delta^{13}\text{C}$ shows a negative,

but weak, trend with increasing temperature across Antarctic and subantarctic locations (Supporting Information, Fig. S1a). Similarly moss species $\delta^{13}\text{C}$ 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 widely distributed species *S. uncinata*, across multiple locations.

We suggest that the influence of growth form and temperature on $\delta^{13}\text{C}_{\text{BULK}}$, 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}_{\text{BULK}}$ 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 $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ as an indicator of growth water environment can be relied upon.

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243 **Supporting Information**

244 **Table S1**

245 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
<i>Temperature trend</i>	Significant rise in mean temp of 1.3°C between 1960s and the 1990s. Furthermore, annual temps were warmer and more stable between 1976 and 2008 ($4.84 \pm 0.27^\circ\text{C}$) than over the period 1951 to 1975 ($4.30 \pm 0.44^\circ\text{C}$).	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.
<i>References</i>	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., (2004)

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252 **Table S2**
253 Bulk carbon isotope ($\delta^{13}\text{C}$) ranges and calculated means of moss species from subantarctic (SOI) and maritime
254 Antarctic locations. Data are from the current study and the literature. (*) indicates $\delta^{13}\text{C}$ value based on a single
255 measurement, other $\delta^{13}\text{C}$ values are based on 2+measurements.

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

<i>Drepanocladus uncinatus</i>	Gibbs Island (SOI)	-24.29*	-24.29*	Galimov 2000
<i>Polytrichum alpestra</i>	Signy Island (SOI)	-25.21*	-25.21*	Galimov 2000
<i>Polytrichum alpinum</i>	Signy Island (SOI)	-25.86*	-25.86*	Galimov 2000
<i>Polytrichastrum alpinum</i>	King George Island, maritime Antarctica	-27.3*	-27.3*	Lee et al., 2009
<i>Polytrichastrum strictum</i>	King George Island, maritime Antarctica	-28.2 to -26	-26.9± 0.8	Lee et al., 2009
<i>Sanionia georgico-uncinata</i>	King George Island, maritime Antarctica	-27.9 to -24	-26.1 ± 1.5	Lee et al., 2009
<i>Sanionia uncinata</i>	Macquarie Island (SOI)	-30.3 to -24	-27.5 ± 2.1	Current study
<i>Schistidium antarctici</i>	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 $\delta^{13}\text{C}_{\text{BULK}}$ of moss growing in subantarctic and maritime Antarctic locations. Data are from the current study and the literature.

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