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A calibration of cellulose isotopes in modern prostrate *Nothofagus* and its application to fossil material from Antarctica

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Abstract

Carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$) in tree rings are widely used to reconstruct palaeoclimate variables such as temperature during the Holocene (12 thousand years ago - present), and are used increasingly in deeper time. However, their use is largely restricted to arboreal trees, which excludes potentially important data from prostrate trees and shrubs, which grow in high latitude and altitude end-member environments. Here, we calibrate the use of $\delta^{13}C$ and $\delta^{18}O$ as climatic archives in two modern species of southern beech (*Nothofagus*) from Tierra del Fuego, Chile, at the southern limit of their current range. We show that prostrate trees are potentially suitable archives for recording climatological means over longer periods (on the order of decades), which opens up these important environments for tree ring isotope analysis. We then apply our new understanding to a remarkable late Neogene (17-2.5 Ma) fossil *Nothofagus* assemblage from the Transantarctic Mountains, Antarctica, representative of a prostrate tundra shrub growing during a period of significant ice sheet retreat. The δ^{13}C of the fossil cellulose was found to be ~4‰ enriched relative to that of the modern tress. This is likely to be due to a combination of a more positive δ^{13}C of contemporaneous atmospheric CO_2 and enhanced water use efficiency at the fossil site. Using the cellulose-δ^{18}O in the fossil wood, we are able to reconstruct precipitation oxygen isotopes over the Antarctic interior for the first time for this time period. The results show that $\delta^{18}O_{precip}$ over Antarctica was -16.0±4.2‰, around 12‰ enriched relative to today, suggesting changes in the hydrological cycle linked to warmer temperatures and a smaller ice sheet.

Keywords: Antarctica; Neogene; Sirius Group; tree ring isotopes, precipitation

1 Introduction

Tree ring stable isotope analysis is a powerful and widely-used tool for palaeo-climatic reconstructions (Cernusak and English, 2014; Gessler *et al.*, 2014). It can provide rare insights into terrestrial palaeo-climate and environmental evolution at high temporal resolution, providing information on temperature (Gagen *et al.*, 2007; Naulier *et al.*, 2014; Lavergne *et al.*, 2016, 2018), precipitation (Cullen and Grierson, 2009; Xu *et al.*, 2016), drought (Kress *et al.*, 2010; Labuhn *et al.*, 2016), and large-scale atmospheric circulation patterns (Xu, Sano and Nakatsuka, 2013; Grießinger *et al.*, 2018).

A key source of information in much of this work is the oxygen isotopic composition of tree ring cellulose ($\delta^{18}O_{cell}$). The theory on the underpinning variables controlling $\delta^{18}O_{cell}$ is relatively well developed, albeit with large uncertainties and knowledge gaps e.g. (Gessler *et al.*, 2014; Treydte *et al.*, 2014). The relationship between these variables and $\delta^{18}O_{cell}$ can be described by various numerical models and used to investigate oxygen isotope variations in multiple settings (Roden and Ehleringer, 2000; Farquhar and Gan, 2003; Ogée *et al.*, 2003, 2009; Danis *et al.*, 2012; Lavergne, Gennaretti, *et al.*, 2017). Cellulose oxygen isotopes are governed by a complex array of factors, including source water isotopic composition (itself a result of precipitation isotopes, soil residence time, and evaporative effects); leaf water enrichment due to transpiration (Yakir and Sternberg, 2000); fractionation between leaf water and carbonyl oxygen (Sternberg and DeNiro, 1983; Sternberg and Ellsworth, 2011); and other oxygen exchange processes between organic compounds and surrounding water, for example during remobilisation of organic matter or cellulose biosynthesis (e.g. Hill *et al.*, 1995; Sternberg *et al.*, 2006; Gessler *et al.*, 2007; Offerman *et al.*, 2011; Nabeshima *et al.*, 2018). The underpinning link with source water oxygen isotopes means that tree-ring cellulose $\delta^{18}O$ can be used to reconstruct the oxygen isotopic composition of precipitation. This in itself is a function of precipitation amount, altitude, temperature, residence time in the atmosphere, distance from moisture source and transport patterns (Dansgaard, 1964; Sime *et al.*, 2009; Aggarwal *et al.*, 2012). Cellulose $\delta^{18}O$ can therefore be used as a proxy for reconstructing global and regional hydrological change, for example, changes in basinal water regimes (Brienen *et al.*, 2012) or large-scale atmospheric circulation patterns (Baldini *et al.*, 2008; Zhu *et al.*, 2012).

Carbon isotopes in tree-ring cellulose ($\delta^{13}C_{cell}$) also have utility as a palaeoclimatic proxy. In general, $\delta^{13}C_{cell}$ is controlled by the $\delta^{13}C$ of atmospheric CO_2 (McCarroll and Loader, 2004; Treydte *et al.*, 2007); atmospheric CO_2 concentrations (Beerling, 1996; Köhler *et al.*, 2010; Battipaglia *et al.*, 2013), along with other factors that affect stomatal conductance including soil moisture and atmospheric vapour pressure deficit; and factors that control photosynthetic capacity such as nutrient availability and irradiance (Ehleringer *et al.*, 1986; Farquhar, Ehleringer and Hubick, 1989; Cernusak *et al.*, 2007; Cernusak, Winter and Turner, 2009). A range of downstream metabolic processes also play a role in shaping $\delta^{13}C_{cell}$, including post-carboxylation fractionation, phloem loading and transport, and respiratory isotope fractionation (Gessler *et al.*, 2009; Priault, Wegener and Werner, 2009; Werner and Gessler, 2011; Werner *et* *al.*, 2011).

Both carbon and oxygen tree ring isotopes are increasingly being applied to older time periods of up to 53 Ma as more fossil plants with adequate preservation are being recovered (Jahren and Sternberg, 2008; Schubert and Jahren, 2011; Schubert *et al.*, 2012; Wolfe *et al.*, 2012; Hare *et al.*, 2018). One particular advantage of this growing dataset is the ability of tree ring isotopes to reconstruct climatic parameters that are much harder to access through marine sediments. These include environmental geochemical signals like precipitation isotopes (Ballantyne *et al.*, 2006; Jahren and Sternberg, 2008; Jahren *et al.*, 2009) and atmospheric carbon isotopes (Arens, Jahren and Amundson, 2000; Jahren *et al.*, 2001). A notable example is the rich treasure trove of exceptionally well-preserved Eocene and Pliocene fossil wood from multiple kimberlite deposits in the Canadian High Arctic. These fossil recoveries have revealed unique details about Eocene and Pliocene palaeoclimate and hydrological cycling through their stable isotope records, such as reconstructing terrestrial temperatures and the isotopic composition of precipitation as well as providing insights into high latitude climate variability (Ballantyne *et al.*, 2006, 2010; Jahren and Sternberg, 2008; Jahren *et al.*, 2009; Csank *et al.*, 2011; Wolfe *et al.*, 2012).

In this study, we apply tree ring isotope analysis to a unique suite of fossil prostrate or *krummoltz Nothofagus* trees recovered from the mid-late Neogene (~17 – 2.5 Ma) Sirius Group deposits at the Oliver Bluffs in the Transantarctic Mountains, Antarctica (85°07'S, 166°35'E; Webb and Harwood, 1987, 1993; Francis and Hill, 1996; Hill, Harwood and Webb, 1996). The plants were deposited at a similar latitude to today (Lawver and Gahagan, 2003) and represent a period of significant Antarctic Ice Sheet retreat, where warming of the continent allowed a tundra-like shrub to grow 480 km from the South Pole.

Based on both geochemical (Rees-Owen *et al.*, 2018) and macrofossil-derived (Francis and Hill, 1996; Ashworth and Cantrill, 2004) palaeothermometers, continental summer temperatures during the trees' lifetimes were ~5°C, implying a weakened latitudinal temperature gradient compared to the present day, where the mean temperature in December is -3.4°C (McMurdo Station; 77°51'S, 166°40'E). Shallower gradients are also supported by vegetation and marine proxy-based reconstructions, indicating, for example, a reduction of ~5.5°C in the meridional temperature gradient during the early Pliocene relative to today (Brierley *et al.*, 2009; Pound *et al.*, 2012).

The age of these sediments has been the subject of a lengthy debate, relating to the nature of the East Antarctic Ice Sheet under warmer-than-present conditions (Barrett, 2013). Biostratigraphical dating of the plant fossils by association with late Pliocene marine diatoms (Webb *et al.*, 1984; Harwood, 1986) suggests the incursion of seaways deep into the Antarctic interior and indicates a dynamic ice sheet as late as 3 million years ago. This relatively young age for the plant fossils has been challenged by suggestions that the diatoms represent wind-blown contamination from the open ocean (Burckle and Potter, 1996; Stroeven, Prentice and Kleman, 1996). Furthermore, cosmogenic exposure dating of nearby moraines indicates these sediments are much older (at least 5 Ma, but possibly as old as 17 Ma; Ackert, Jr. and Kurz, 2004) and therefore that the ice sheet has been a stable climatic feature since the mid-Miocene. Evidence for a periodically reduced ice sheet accompanied by vegetation along the margins exists for the mid-Miocene (17-15 Ma; Warny *et al.*, 2009; Feakins, Warny and Lee, 2012; Griener *et al.*, 2015; Gasson *et al.*, 2016; Levy *et al.*, 2016). Increasingly both modelling (Dolan *et al.*, 2011; Austermann *et al.*, 2015; Pollard, Deconto and Alley, 2015; Pollard and Deconto, 2016) and data (Fielding *et al.*, 2012; Cook *et al.*, 2013; Ohneiser *et al.*, 2020) studies also suggest that at least partial EAIS retreat occurred during the Pliocene, allowing a tundra shrub to grow around 4.1 Ma. These competing scenarios pose a challenge to dating these fossils. Nevertheless, the fossiliferous bed clearly represents a period of significant East Antarctic Ice

Sheet (EAIS) retreat in response to warming temperatures (Mercer, 1986; Francis and Hill, 1996). Our data will therefore give novel insight into past Antarctic climate change during a vital period in its glacial history.

To date, the vast majority of tree ring stable isotope studies have been applied to trees with an arboreal habit. Prostrate trees (where stems grow horizontally to avoid harsh conditions such as freezing winds e.g. *Salix arctica* in the High Arctic and the fossil plants considered in this study) and shrubs are increasingly used in modern dendrochronological studies (Woodcock and Bradley, 1994; Hantemirov, Shiyatov and Gorlanova, 2011; Buras and Wilmking, 2014), where they can provide vital information on past climate for tree-less regions such as those at high latitude or altitude, and deserts. However, to our knowledge, no studies using tree ring isotopes in prostrate plants to reconstruct past climate exist, so there is uncertainty over the extent to which isotope theory developed for arboreal tree rings holds true for *krummholz*-type plants. Therefore, the objective of the first part of this study is to calibrate the use of tree ring isotopes (δ^{13}C and δ^{18}O) in high latitude prostrate trees for climatic reconstructions using plants from Isla Navarino, Chile, where two deciduous southern beech (*Nothofagus*) species grow in both arborescent and prostrate form in a subpolar forest environment at the southern limit of their range. The objective of the second part is to apply this new knowledge to our fossil *Nothofagus* trees to enhance our understanding of how the Antarctic Ice Sheet has behaved during past warm periods of Earth's history.

2 Materials and methods

2.1 Oliver Bluffs; fossil site

The fossil wood was sampled from a sedimentary succession at Oliver Bluffs in the Dominion Range of the Transantarctic Mountains (85°07'S, 166°35'E), which forms part of the Sirius Group sediments (Fig. 1). The fossil plant material occurs within one main bedding horizon in the central part of the exposure at Oliver Bluffs, on the eastern side of the upper valley of the

Beardmore Glacier. The present elevation is approximately 1760 m above sea level, but deposition likely occurred at a much lower altitude (Webb and Harwood, 1993; Ackert, Jr. and Kurz, 2004). The sedimentary sequence consists of glacial diamictites, and are thought to be lodgement tills deposited by the ancestral Beardmore Glacier during glacial advance and retreat (McKelvey *et al.*, 1991). The fossiliferous bed containing fossil wood and leaves comprises poorly-sorted sandstones with silt lenses, representing an outwash deposit, in places burying poorly-developed glacial soils on a braided outwash plain (Ashworth and Cantrill, 2004). We envisage sporadic accretion of sediment over the plain, such that the fossiliferous bed is spatially heterogeneous, but as a whole is representative of a significant portion of the ice sheet retreat event (Rees-Owen *et al.*, 2018).

The fossil wood fragments were first described as *Nothofagus* (Carlquist, 1987) and later identified as *Nothofagus beardmorensis* and are dated to between 17 and 2.5 Ma (Hill and Jordan, 1996; Hill, Harwood and Webb, 1996). Leaf remains and tree ring analyses suggest that these were deciduous prostrate shrubs, very similar to the *krummholz N. pumilio* and *N. antarctica*, which grow at the treeline in Tierra del Fuego, Chile (Francis and Hill, 1996). Due to the small ring size (<100 μm) and friability of the material, fossil wood fragments were sampled for isotope analysis in bulk or by isolating individual rings where possible, so our measurements are averages over multiple years and up to several decades. The necessity of combining multiple rings together for the analysis of the fossil wood material sets the context for the modern part of our study in which we stress the interpretation of data on decadal rather than annual timescales.

2.2 Isla Navarino; modern analogue site

Isla Navarino (55°56'S, 67°37'W; Fig. 1) is part of the Magellanic subpolar forests ecoregion which stretches west of the Andes down to Tierra del Fuego, Chile. The island has a maritime climate, with mean annual temperatures of 6 °C, average summer highs of 10°C and winter averages of 2°C. Cool windy conditions prevail year round; Mean Annual Precipitation (MAP) is 400-500 mm, which is uniformly distributed throughout the year. The island vegetation is characterised by Magellanic forest dominated by *Nothofagus* trees to the north, and Magellanic moorland to the south.

The overall intention of this study is to ascertain whether the Antarctic fossil trees can be used for tree ring isotope work, so we designed our sampling strategy for the modern plants to mimic this where possible, including limiting our study to *Nothofagus* only. Wood cores and rounds from branches (for prostrate trees) from 31 living trees were collected at five sites on Isla Navarino during the austral summer of 2013. Three species of *Nothofagus* trees grow on the island, one evergreen species (*N. betuloides*) and two deciduous species (*N. antarctica* and *N. pumilio*). Because the fossil *Nothofagus* from the Sirius Group sediments are deciduous (Hill, Harwood and Webb, 1996), cores were taken from two deciduous *Nothofagus* species over an altitude transect from near sea-level to the treeline (~600 m) at 5 sites (Table 1; Fig. 1). Over the transect, *Nothofagus* ranged in habit from arborescent (single stem and generally greater than 4 m in height) to *krummholz* form (i.e. prostate, with a small trunk or stem and multiple branches lying horizontally upon the ground). Species were identified by leaf character (Moore, 1983) and sampled during the height of austral summer 2013 (January), when the trees were in full leaf. Arborescent trees were cored at chest height (~130 cm above the ground) using an increment wood corer with a diameter of 5 mm. Prostrate individuals were sampled from primary branches in order to match sampling from the fossil trees. Two cores or rounds were sampled per individual tree and the cores and rounds were air-dried; cores were stored in plastic straws. Rounds were sanded with progressively fine sandpaper, and the surface of the tree-ring cores were cut using a core-microtome to improve ring visibility.

The core samples were dated to the calendar year of their formation and cross-dated using the techniques described in (Stokes and Smiley, 1968). These were then statistically tested using the programme COFECHA (Holmes, 1983; Grissino-Mayer, 2001) and chronologies were constructed using ARSTAN. As the austral growing season overlaps two calendar years, rings were assigned to the year when ring growth began (i.e. the last complete ring taken for each core in January 2013 was dated to austral summer 2011, as the 2012-2013 ring was still incomplete at the time of sampling).

A 30 year sequence was isolated for isotopic analysis covering the period 1981-2011. This sequence length was chosen to roughly match the available tree ring spans of the fossil trees sampled here. Tree rings are composed of earlywood and latewood: the former comprises large thin-walled cells made of stored photosynthates from the previous year and the latter comprises thicker-walled cells formed during summer. Therefore to sample at true annual resolution, it has been suggested that only latewood should be taken (Switsur *et al.*, 1995). However, the rings in the prostrate plants in this study were too small to obtain sufficient latewood, so the entire ring was sampled each time; this approach has been used successfully to reconstruct temperature in the same region (Lavergne *et al.*, 2016). Isotope ratios were measured separately for each year and each tree. There are multiple missing years in the isotope chronologies where rings were too small to extract sufficient cellulose for analysis.

Chronologies at annual resolution require the construction of chronologies that are statistically representative of the variability of the site. An Expressed Population Signal (EPS; Wigley, Briffa and Jones, 1984) was calculated for each site's δ^{18}O and δ^{13}C chronologies. This is a measure of how well a chronology constructed from a finite number of trees represents the hypothetical perfect or true chronology; a value of 0.85 is generally considered to be an acceptable confidence level. On the whole, EPS is highly sensitive to the number of trees in the chronology. In this study, the EPS for each site was low (particularly for δ^{13}C) (0.65 – 0.87 for δ^{18}O; 0.46 – 0.76 for δ^{13}C), suggesting that a greater sample size is needed to be representative of the whole sample site, particularly for δ^{13}C, which generally exhibits lower EPS (Daux *et al.*, 2018).

Because we are not intending to develop a detailed chronology for Isla Navarino, but instead test whether tree ring isotopes are broadly applicable to our prostrate fossil trees, we judge that this is adequate for the purposes of this study.

Soil and root samples were also collected, along with water from a stream network covering the altitude transect in order to estimate source water $\delta^{18}O$. Soils were sampled from 50 cm depth around the roots of three trees from each of the five sites (where 90% of *Nothofagus* forest root mass is situated; Schulze *et al.*, 1996). Root samples were taken from at least one tree at three of the five sites. Roots and soils were wrapped in cling film, stored in multiple airtight bags and frozen until required for water extraction. Source water samples were taken from seven fast-flowing streams and one lake, covering the entire altitudinal transect, filtered (0.2 μm), and stored in McCartney vials.

2.3 Sample preparation and isotopic analysis

Except where otherwise indicated, the following procedures were all carried out in the University of Leeds Cohen Geochemistry laboratories in the School of Earth and Environment, 2013 - 2016.

Oxygen isotope ratios are expressed as $\delta^{18}O$; where delta notation is the conventional notation used for the ratio of isotopes (e.g. $^{18}O/^{16}O$) in a sample (R) relative to a standard (R_{STD}) such that $\delta = (R/(R_{STD} - 1)1000,)$ reported in per mil (‰). Results are reported with respect to Vienna Standard Mean Ocean Water (VSMOW). Carbon isotope ratios ($^{13}C/^{12}C$) are expressed as $\delta^{13}C$ and reported relative to the Vienna Pee Dee Belemnite standard.

2.3.1 Preservation of fossil material

Exceptional preservation of the fossil *Nothofagus* utilised in this study is well documented (Francis and Hill, 1996), and is supported by scanning electron microscope imaging (Fig 1D), which shows excellent retention of wood fibres. Although it is clear that some degradation of vessels has occurred, this should not impact the isotopic signal of the remaining cellulose; cellulose extracted from fossil trees significantly older than those used in this study (up to 53 Ma; (Wolfe *et al.*, 2012; Hook *et al.*, 2014, 2015; Staccioli, Santoni and Pizzo, 2014) was extracted in low yield (<5%; Hook *et al.*, 2015) indicating a high degree of cellulose degradation, but showed no signs of isotopic alteration. Mineral contaminants in the form of microcrystalline calcite were detected in the Sirius Group fossil trees using energy dispersive X-ray spectroscopy, which could affect both $\delta^{18}O$ and $\delta^{13}C$, but the delignification step during extraction is performed below pH 5, which removes all calcite. After extraction, cellulose was recovered as a white fluffy material (5 - 30% yield), which is a clear indication that cellulose is well-preserved and hence the fossil material is appropriate for isotope analysis.

2.3.2 Cellulose isotope measurements

Cellulose was extracted from both modern and fossil samples using batch extraction equipment described by (Wieloch *et al.*, 2011). To summarise, ground wood samples were heated in aqueous NaOH solution (5%, 2 hours, 60°C, repeated twice) to remove tannins, resins and fatty acids. Samples were then heated (60°C) in acidified $NaClO_2$ (via glacial acetic acid; 7.5%, pH 4-5) for 10 hours; this step was repeated four times to ensure complete delignification. Finally, we used a solution of NaOH (17%; 60°C, 2 hours) to remove hemicelluloses, leaving α-cellulose for analysis. Cellulose samples were homogenised using a Retsch MM301 Mixer Mill, then freeze-dried for 24 hours to remove ambient water. Samples were stored in Eppendorf vials and kept in a desiccator for >24 hours prior to isotope analysis.

In order to measure $\delta^{18}O_{cell}$, the milled, freeze-dried cellulose samples were weighed, packed into silver capsules and pyrolysed at 1450°C. Oxygen isotope ratios were measured using an elemental analyser with a purge and trap column (Elementar vario PYRO cube), coupled to an Isoprime isotope ratio-mass spectrometer. Ratios of $^{18}O/^{16}O$ were converted to $\delta^{18}O_{VSMOW}$ with a one point linear calibration using IAEA-601 (benzoic acid; $\delta^{18}O = 23.15 \pm 0.3$‰) with reference to cellulose from Sigma-Aldrich, UK (Lot#SLBD2972V; hereafter Leeds Sigma cellulose). The Leeds Sigma cellulose was analysed at the University of Leeds against IAEA-CH-3 cellulose, assuming $\delta^{18}O = 31.9 \pm 0.5$‰ (Hunsinger, Hagopian and Jahren, 2010) and assigned a value of 29.2±0.2 . Standards were included at an interval of every twelve samples. Within-run reproducibility of an internal check standard was ±0.37‰. For $\delta^{13}C$ analysis, extracted cellulose samples were weighed and packed into tin capsules. Carbon isotope ratios were measured using an Elementar vario PYRO cube elemental analyser coupled to an Isoprime mass spectrometer. The encapsulated samples were combusted at 1150°C in pure oxygen. Ratios of $^{13}C/^{12}C$ were calibrated to the international VPDB scale using in-house urea and C4 sucrose. These were assigned values of -46.83±0.22‰ and -11.93±0.24‰, respectively after calibration using six replicates of each of the following international standards: IAEA-LSVEC (-46.479‰), IAEA-CH7 (-31.83‰), IAEA-CH6 (-10.45‰) and IAEA-CO1 (+2.48‰). The precision obtained for repeat analysis was better than ±0.2‰ (σ).

2.3.3 Water isotope measurements

Water was extracted from roots and soils by cryogenic vacuum distillation, following the procedure detailed by West, Patrickson and Ehleringer (2006). Extracted samples, along with stream waters, were stored frozen until they were measured for water isotope ratios at the School of Environmental Sciences, University of East Anglia, UK. The $^{18}O/^{16}O$ ratios were analysed using a Picarro L1102-i cavity ring-down spectroscopy analyser with a CTC Analytics autosampler. Each sample was injected and measured 6 times using 2.5 µl of water for each injection. Together with the samples, two secondary international standards (USGS 64444 and USGS 67400) and one internal laboratory standard (NTW – Norwich tap water) were measured, each injected 10 times in order to minimize memory effects. Final isotopic compositions were calculated using the calibration line based on the secondary international standards and reported in permil units with respect to V-SMOW on the V-SMOW – SLAP scale. The precision of the measurements is 0.1 ‰ for $\delta^{18}O$.

The isotopic composition of plant source water for the modern *Nothofagus* in this study was constrained by measuring $\delta^{18}O$ of soil waters ($\delta^{18}O_{soil}$) for the five sites, which ranged between -13.1±0.73‰ and -10.6±1.17‰ (1 σ; grand mean = -11.9±0.89‰; n=16; Table 1). Oxygen isotopes from eight streams and lakes across the sampling transect ($\delta^{18}O_{stream}$), ranged between -11.1‰ and -9.8‰ (mean = -10.8±0.41‰). Root water extracted from *Nothofagus* trees at three sites (mean = -10.5±0.54‰, n=4) was isotopically similar to $\delta^{18}O_{stream}$ and $\delta^{18}O_{precip}$, indicating that plants took up water from an annually integrated precipitation signal.

The $\delta^{18}O$ data presented here only represent one year's precipitation. We also used temperature, precipitation and precipitation $\delta^{18}O$ data from the nearby Global Network of Isotopes in Precipitation (GNIP) station at Ushaia, Argentina (54°46'48" S; 68°16'48" W), approximately 50 km away, in order to take into consideration summer and winter seasonal precipitation in this study, noting that there are a number of missing years for the data set; a more complete dataset is available from Punta Arenas but this station is significantly further away. Mean summer precipitation for Ushaia was -9.9±0.9‰; mean winter precipitation was -11.92±0.75‰, which is not statistically different from the mean soil water $\delta^{18}O$ (p<0.001).

2.3.4 Modelling $\delta^{18}O_{source}$

There are multiple models of varying complexity linking these parameters and it is not clear whether more complex models provide better predictions than simpler ones. For the purposes of this study, we used a relatively simple model given by eq. 1 (Anderson *et al.*, 2002), which was chosen because there are only two unconstrained parameters (relative humidity, RH, and the fraction of leaf water not subject to fractionation, *f*). We acknowledge that there are more complex process-based and mechanistic models described in the literature, but consider that the use of more complex models linking $\delta^{18}O_{source}$ with $\delta^{18}O_{cell}$ (Roden, Lin and Ehleringer, 2000; Danis *et al.*, 2012) would require making assumptions about a larger number of parameters which are difficult to constrain in deep time, for example amount of precipitation and daily max and min temperatures. The Anderson model has been used in multiple studies to reconstruct past precipitation isotopes (Csank *et al.*, 2011; Wolfe *et al.*, 2012; Hook *et al.*, 2015).

$$\delta^{18}O_{source} = \delta^{18}O_{cell} - (1 - f)(1 - RH)(\varepsilon_e + \varepsilon_k) - \varepsilon \qquad (1)$$

where ε is the biological fractionation factor associated with the formation of cellulose (+27±3‰; (Sternberg and DeNiro, 1983), ε_e is the equilibrium liquid-vapour fractionation for water and approximates $\delta^{18}O$ of atmospheric vapour (assumed here to be 11‰; Majoube, 1971) and the subscript *source* denotes source water. The kinetic liquid-vapour fractionation (ε_k) is dependent on leaf morphology and boundary layer vapour transport conditions; broad-leaf trees have quasi-laminar boundary layer conditions so ε_k = 21‰ (Buhay, Edwards and Aravena, 1996). The parameter *f* is the fraction of leaf water not subject to evaporation (Allison, Gat and Leaney, 1985) and also includes the isotopic alteration of carbon-bound oxygen via exchange with stem water (Roden and Ehleringer, 1999).

We tested the assumptions made by Anderson et al. (2002) using measured $\delta^{18}O_{cell}$ from the modern analogue trees as input for the model (with RH = 0.7, *f* = 0.2 as in Allison et al., 1985) and compared the results against measured $\delta^{18}O_{source}$ (i.e. soil and stream water) $\delta^{18}O_{root}$ from Isla Navarino, and the GNIP precipitation data from Ushaia. In order to apply the model to fossil *Nothofagus*, we applied a large range of values for RH that are consistent with measurements from high latitude modern analogue sites such as Isla Navarino (0.5 - 0.85) and using a random number generator with uniform distribution, we sampled between these constraints (n=10000) to provide an estimate of the possible range of $\delta^{18}O_{precip}$.

3. Results and discussion

3.1. Oxygen and carbon isotope ratios in modern Nothofagus

Oxygen and carbon isotope ratios in modern *Nothofagus* trees over a range of morphologies were measured to provide a first order check on the ability of fossil prostrate *Nothofagus* plants to record long-term climate and environmental variables. Mean $\delta^{18}O_{cell}$ for all sites over the sample period (1981-2011) ranged between 24.1‰ and 26.9‰. There was no statistically significant difference between the two Nothofagus species (p>0.7; Student's unpaired *t*-test). However, there is also no statistically significant difference between sites for mean $\delta^{18}O_{cell}$, and therefore for altitude and morphology (i.e. prostrate or arboreal form), indicating that morphology does not impact absolute $\delta^{18}O_{cell}$ integrated over multiple tree rings. Intriguingly, prostrate trees in this study exhibit much lower inter-tree variability than their arborescent counterparts (σ = 2.1‰ and σ = 0.8‰, for arboreal and prostrate morphologies, respectively (Fig. 3)). Prostrate plants are more aerodynamically decoupled from the atmosphere, and retain tight control over their microclimate (Barrera *et al.*, 2000; Korner, 2003), which may reduce inter-tree variability in transpiration.

Mean $\delta^{13}C_{cell}$ for each site ranged between -27.2‰ and -26.7‰, (grand mean = -26.6±0.7‰), which is consistent with typical values for C3 land-plants (O'Leary, 1988). Mean inter-tree variability was low (σ range = 0.6 - 0.8‰) and did not vary with altitude or morphology. In this case, $\delta^{13}C_{cell}$ variability may be dominated by carbon assimilation rather than stomatal conductance (in support of findings of Farquhar, Barbour and Henry, (1998) and Scheidegger *et al.*, (2000) for example). A dominant stomatal conductance signal would lead to co-varying carbon and oxygen isotope ratios with morphology (Lavergne, Daux, *et al.*, 2017; Guerrieri *et al.*, 2019).

The low EPS in this study means our chronology is inappropriate for studying climate variations at interannual resolution, and would need to be updated with more trees if that were the purpose of the study. However, this does not prevent us using the data to understand a longer-term integrated climate signal; the low inter-tree variability in the prostrate plants in particular suggests they may function well as a record of climate information integrated over longer timescales and we test this hypothesis using a physiological model below. This is particularly pertinent to the fossil plants in this study, where the tree ring widths are extremely narrow and do not provide sufficient material for analysis at annual resolution; data from the fossil plants is integrated over the entire individual plant.

3.2 Carbon isotopes in fossil Nothofagus

Mean $\delta^{13}C_{cell}$ was -22.6±1.9‰ (1 σ). The inter-tree variability here is much larger than in either the arboreal or prostrate plants from Isla Navarino (-26.6±0.7‰), which again is consistent with the dataset spanning millennial timescales. This range of values is significantly enriched by ~+4‰ ($p<0.001$) relative to the mean values seen in the modern *Nothofagus* trees (Fig. 2).

Scarring on the bark (Francis and Hill, 1996) of the fossil plants implies strong winds and paleosol analysis suggests that MAP was 120-220 mm (Retallack, Krull and Bockheim, 2001), which is considerably lower than the MAP on Isla Navarino (400-500 mm). This would lead to enhanced water stress, although fossil *Nothofagus* leaves associated with the wood fragments are large in size indicating that the plants were not living in a marginal habitat (Hill et al., 1996) and thus any water stress could not have been too severe. For both fossil tree age scenarios, atmospheric CO_2 was equal to, or greater than present day, at ~400 ppm for the Pliocene, and exceeding 500 ppm for the warmest periods of the mid-Miocene (Pagani *et al.*, 2010; Holbourn *et al.*, 2015; Levy *et al.*, 2016). Both of these factors would lead to enhanced water-use efficiency, reducing stomatal conductance and hence enriching $\delta^{13}C_{cell}$.

Additionally, Tipple, Meyers and Pagani, (2010) (Tipple, Meyers and Pagani, 2010) find that $\delta^{13}C$ of atmospheric CO_2 was higher than present day for both the Pliocene (between around -6.7 and -6‰) and mid-Miocene (between around -5.7 and -5‰), compared to between -8.5 and -7.5‰ for the present day (Keeling *et al.*, 2017). This increase in the baseline $\delta^{13}C$ in combination with enhanced water-use efficiency, is sufficient to explain the large enrichment we see between modern and fossil $\delta^{13}C_{cell}$ data.

3.3. Source water $\delta^{18}O$ in modern *Nothofagus*

There was no significant trend in $\delta^{18}O$ of measured soil, root or stream waters with altitude, which is most likely because of the small altitude range covered in this study (0-600 m). Sites 2 and 3 $\delta^{18}O_{soil}$ are statistically different from each other ($p<0.05$; one way ANOVA with post-hoc Tukey test) and site 2 is also significantly depleted relative to the stream and root water, suggesting an increased contribution from winter precipitation to soils. From these observations we infer that plant source water $\delta^{18}O$ ($\delta^{18}O_{source}$) can be treated as $\delta^{18}O_{precip}$, where $\delta^{18}O_{precip}$ is controlled by latitude, condensation temperature and precipitation amount (Dansgaard, 1964).

3.4 Source water $\delta^{18}O$ prediction from modern cellulose $\delta^{18}O$

We now test whether $\delta^{18}O_{precip}$ can be reconstructed from $\delta^{18}O_{cell}$, using the model from Anderson et al., (2002).

The chosen model under-predicted $\delta^{18}O_{source}$ by between 0.2‰ and 2.9‰ (mean for all sites =

1.5‰; Fig. 3). This could be due to model parametrization; we chose a value of exactly 27‰ for the biological fraction factor ε, but another value within the accepted range of 24-30‰ could equally be chosen (as in Anderson et al., 2002 and demonstrated by the *x*-axis error bars). Moreover, modelled $\delta^{18}O_{source}$ was not statistically different from $\delta^{18}O_{root}$, $\delta^{18}O_{stream}$ or GNIP summer precipitation, indicating that the model works well for predicting $\delta^{18}O_{precip}$ from measured $\delta^{18}O_{cell}$ in *Nothofagus*. We now apply the model to the fossil *Nothofagus* in order to calculate ancient $\delta^{18}O_{precip}$.

3.5. Reconstructing ancient precipitation $\delta^{18}O$ from fossil Nothofagus

Mean $\delta^{18}O_{cell}$ for the fossil plants was 20.3±3.0‰. The inter-tree variability is similar in magnitude to that seen in modern trees, but is greater than the inter-tree variability seen in the prostrate plants of this study. It seems likely that these data capture both significant temporal variability and climate variability. It is important to note that here, we are treating all fossils as being geologically contemporaneous as they were all collected from the same bed, but it is highly likely that our data may span multiple millennia. Ice sheet fluctuations during both the mid-Miocene and Pliocene occurred at orbital timescales (Greenop *et al.*, 2014; Patterson *et al.*, 2014); therefore the duration represented by the fossils must be less than 100 kyr, but long enough for poorly developed soils to form and woody plants to colonise the area. This is consistent with the larger variability in the fossil data compared to the modern. Mean $\delta^{18}O_{cell}$ for the Sirius Group plant is significantly depleted by ~5‰ (p<0.001) relative to the mean of the modern *Nothofagus* trees from Isla Navarino (25.5±1.5‰). Broadly, there are two major controls on $\delta^{18}O_{cell}$, which could cause such an offset: evapotranspiration rates (controlled by relative humidity and stomatal conductance) and $\delta^{18}O$ of the plant's source water (McCarroll and Loader, 2004). From the modern data, we assume that plant source water is equal to precipitation $\delta^{18}O$ within the uncertainty of precipitation variability. In addition to latitude, precipitation amount, and temperature, there are further processes that could modify this signal, including evaporation from soil or plants using groundwater as a moisture source. Depletion could result from a large decrease in stomatal conductance caused by increased vapour pressure deficit reducing evapotranspiration from leaves. However, vapour pressure deficit across southern Chile is already relatively low (<0.5 kPa; Du *et al.*, 2018) and it is unlikely that there would have been significant decreases in vapour pressure deficit for Antarctica when summer temperatures are not predicted to have been much lower (Rees-Owen *et al.*, 2018). Alternatively, decreased $\delta^{18}O_{cell}$ could be caused by a difference in $\delta^{18}O_{precip}$, which is consistent with the higher palaeolatitude of the fossil plants (85°S for the Sirius Group, 54°S for Isla Navarino). We test this hypothesis using the physiological model for $\delta^{18}O_{precip}$ from Anderson et al, (2002). Using this approach, we calculate that mean continental Antarctic palaeoprecipitation was -16±4.2‰ (1 σ; ranging between -26‰ and -3.5‰). Since $\delta^{18}O_{cell}$ is strongly modified by ambient relative humidity, the large range in our results is consistent with the conservative (i.e. wide) humidity range used in this study.

In the present day, $\delta^{18}O_{precip}$ over East Antarctica is highly variable, ranging from -55‰ at the highest elevations and furthest from the coast, to -25‰ near sea level at lower latitudes <75°S (Masson-Delmotte *et al.*, 2008). However, there is considerable uncertainty surrounding the palaeoaltitude of the *Nothofagus* fossils sampled in this study (Ackert, Jr. and Kurz, 2004), which makes it difficult to provide context for the reconstructed $\delta^{18}O_{precip}$ values. We therefore compared our record to measured

Antarctic $\delta^{18}O_{precip}$ from sites above 75°S and less than 700 m above sea level (the height of the timberline on Isla Navarino; Masson-Delmotte et al., 2008), representing a reasonable habitat range. Reconstructed $\delta^{18}O_{precip}$ was significantly enriched by ~+12‰ relative to modern $\delta^{18}O_{precip}$ (ancient mean = -16‰, modern mean = -28‰; p <0.001; Fig. 4). Growth experiments have suggested that plant δ^2H (and therefore by extension, δ^{18}O) can be significantly enriched in plants grown under continuous light, analogous to the polar light regime (Yang *et al.*, 2009). Therefore, part of the enrichment in the Sirius Group specimens could be accounted for by the continuous light regime experienced by the Antarctic plants during the growing season, which would increase $\delta^{18}O_{cell}$ via continuous transpiration, as opposed to the light regime on Isla Navarino, where plants undergo a diurnal transpiration-respiration cycle. However, the plants used by Yang et al. (2009) have a relatively high transpiration rate because of the relatively warm growing temperatures used in their experimental study. We suggest that the transpiration rate for the Sirius Group plants would likely be much lower because of the cold summer temperatures (~5°C, compared to ~20°C in the environment used by Yang et al., 2009). Furthermore, *Nothofagus* have been documented as having significantly tighter stomatal control of transpiration than co-existing conifers (Fernández, Gyenge and Schlichter, 2009), as used by Yang et al. (2009). Therefore it seems likely that there is much lower enrichment due to continuous light in the Sirius Group fossils (see Supplementary Information for further discussion).

Our result has implications for regional and global climate during periods of ice sheet retreat in the Neogene. A significant enrichment in precipitation isotopes implies a considerable change in some of the atmospheric processes of the hydrological cycle. Plausible mechanisms include increased temperatures affecting fractionation during condensation, or changes in rainout patterns due to shifts in source moisture region or different atmospheric circulation patterns leading to a shortened vapour transport pathway. As previously discussed, warmer Antarctic temperatures (relative to today) are consistent with multiple contemporaneous terrestrial temperature proxies, which suggest that summer temperatures reached 5°C during the period of study (Ashworth and Kuschel, 2003; Ashworth and Preece, 2003; Ashworth and Cantrill, 2004; Rees-Owen *et al.*, 2018). This result is also consistent with both age scenarios for the site:

during both the mid-Miocene and Pliocene, sea surface temperatures in the Southern Ocean were several degrees warmer than today (Warny *et al.*, 2009; McKay *et al.*, 2012; Clark *et al.*, 2013) and there is evidence for reduced sea ice cover (Whitehead, Wotherspoon and Bohaty, 2005; Warny *et al.*, 2009). However, previous work by Feakins *et al,* (2012) suggests that the relationship between temperature and precipitation isotopes earlier in the Miocene (20 - 15 Ma) on the Antarctic coast was different from the modern, driven by increased evaporation from a warmer Southern Ocean. Similarly, isotopic disequilibrium between vapour and precipitation in modern-day Patagonia has been suggested to explain greater than expected $\delta^{18}O_{cell}$ (Penchenat *et al.*, 2020). This implies that other factors may also influence the hydrological cycle at this time, which is plausible within the context of a warmer Neogene world, where warmer Southern Ocean temperatures could drive an increase in evaporation from high latitude moisture sources. Equally, the smaller ice sheet could well have influenced regional atmospheric circulation patterns, and changes in global atmospheric circulation are documented for the Pliocene (Brierley et al., 2009). These variables are likely to be important for understanding the full significance of our data, but are unconstrained, and a full exploration of hydrological changes is beyond the scope of this study. These questions could be more fully answered through further data collection to reduce proxy uncertainty, and the use of a coupled ocean-atmosphere climate model to investigate hydrodynamic changes.

4. Conclusions

By testing a simple physiological model linking $\delta^{18}O_{cell}$ with $\delta^{18}O_{precip}$ in two species of modern *Nothofagus* plants, which grow in both arboreal and prostrate form, we found that $\delta^{18}O_{cell}$ of prostrate *Nothofagus* faithfully records $\delta^{18}O_{precip}$ at multi- year resolution. Hitherto, most tree ring stable isotope analyses have been applied to trees with an arboreal habit in temperate and tropical environments. Therefore, it was previously unclear whether the assumptions made in tree ring isotope theory hold true for *krummholz*-type plants, such as those from Oliver Bluffs, which feature growth asymmetry that could affect isotopic signals via resource partitioning. Our findings demonstrate that prostrate trees are potentially suitable archives for recording climatological means over longer periods (on the order of decades). This result opens up high latitude and altitude end-member environments in both palaeo and modern times for tree ring isotope analysis.

The carbon isotope composition of cellulose from exceptionally well preserved Neogene fossil wood from the Transantarctic Mountains, Antarctica was ~4‰ more positive than that of the modern samples. This difference is best explained as the result of a more positive value for the δ^{13}C of contemporaneous atmospheric CO_2 and enhanced water use efficiency at the Oliver Bluffs site, although the precise contribution of each of these factors to this signal is unknown.

The oxygen isotopic composition of the fossil wood provides new insights into Neogene hydrological cycling. Our record indicates that during a period of EAIS ice sheet retreat in which small prostrate shrubs colonised the exposed glacial landscape close to the South Pole, the hydrological cycle was markedly different to today with precipitation significantly enriched in ^{18}O by ~12‰ relative to modern precipitation over the continent. While the enrichment may be temperature driven alone, our result correlates well with the result of Feakins et al. (2012), suggesting that moisture source regions may have been different in the past. However, it is not possible to distinguish between these two possibilities, or some combination of both, based on the geochemical data alone.

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Figure Captions

Figure 1: (**A**) Location of sampling sites on Isla Navarino in Tierra del Fuego, Chile. Yellow triangle = marks the location of the GNIP station at Ushuaia; open circles = mark the tree ring sampling sites. (**B**) Fossil wood location at Oliver Bluffs (black filled circle), Transantarctic Mountains, Antarctica. White represents ice; grey shapes are Transantarctic Mountain outcrops. (**C**) Photograph of exceptionally preserved fossil Nothofagus from Oliver Bluffs. (**D**) Scanning Electron Microscope image of fossil *Nothofagus*, demonstrating excellent preservation of wood fibres. (**E**) Prostrate *Nothofagus antarctica* from Isla Navarino.

Figure 2: (**A**) Standard deviation of $\delta^{13}C_{cell}$ (open circles) and $\delta^{18}O_{cell}$ (closed circles) with altitude for Nothofagus from Isla Navarino, demonstrating a decrease in variability for $\delta^{18}O_{cell}$ for prostrate trees. (**B**) Mean $\delta^{18}O_{cell}$ data for modern Nothofagus separated into arboreal and prostrate form, and fossil Nothofagus. (**C**) As panel (**B**) but for $\delta^{13}C_{cell}$.

Figure 3: The relationship between modelled source water $\delta^{18}O$ and measured $\delta^{18}O_{source}$ water for each site (from soils from each of the five sites (circles), roots from three sites (squares) and Global Network of Isotopes in Precipitation $\delta^{18}O_{precip}$ (summer precipitation; diamond). Modelled source water $\delta^{18}O$ was calculated from measured $\delta^{18}O_{cell}$ (modern *Nothofagus*) using the same method as Anderson et al. (2002). Markers give the mean $\delta^{18}O$, y-error bars show the full measured data range, x-errors show the range of modelled $\delta^{18}O_{source}$ if ε were varied within the range given by Sternberg and DeNiro, (1983), and a 1:1 ratio is given by the dotted line for comparison. Modelled data is calculated using RH = 0.7, f = 0.2.

Figure 4: Cellulose $\delta^{18}O$ from the Sirius Group fossil *Nothofagus*, with modelled $\delta^{18}O$ of palaeo precipitation and modern Antarctic snow. Modern measurements from Masson-Delmotte et al. (2008); data restricted to >75°S and below 700 masl. The median is given by the line, the first and third quartiles by the box, and the whiskers denote the full range of data.

Rees-Owen et al, A calibration of cellulose isotopes in modern prostrate Nothofagus and its application to fossil material from Antarctica

Author contribution statement (using CRediT defined terms):

Rhian Rees-Owen: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original draft preparation, Visualisation

Robert Newton: Conceptualization, Methodology, Writing – Review & editing

Ruza F. Ivanovic: Conceptualization, Writing – Review & editing

Jane E. Francis: Conceptualization, Writing – Review & editing

James B. Riding: Writing – Review & editing

Alina D. Marca: Resources, Writing – Review & editing

Declaration of interests

For 'A calibration of cellulose isotopes in modern prostrate Nothofagus and its application to fossil material from Antarctica' by Rhian L. Rees-Owen, Robert J. Newton, Ruza F. Ivanovic, Jane E. Francis, James B. Riding, Alina D. Marca

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Table 1: Summary of sample sites on Isla Navarino with mean site $\delta^{18}O_{soil}$.

Site	Latitude	Longitude	Elevation / m	No. trees	$\delta^{18}O_{soil}$ / ‰	No. soil water replicates
1	54° 56’ 37’’ S	67° 39’ 25’’ W	29	5	-11.5±0.25	3
2	54° 57’ 04’’ S	67° 38’ 58’’ W	97	4	-13.1±0.73	4
3	54° 58’ 33’’ S	67° 40’ 22’’ W	247	5	-10.5±1.17	3
4	54° 59’ 19’’ S	67° 41’ 02’’ W	395	7	-12.0±1.29	2
5	54° 59’ 35’’ S	67° 41’ 04’’ W	527	11	-12.0±0.58	4

Graphical abstract

Rees-Owen et al, A calibration of cellulose isotopes in modern prostrate Nothofagus and its application to fossil material from Antarctica

Highlights

- Prostrate trees record average climate over decadal time periods in tree ring cellulose-$\delta^{18}O$
- Late Neogene Antarctic wood indicates a ~12‰ enrichment in H_2O-$\delta^{18}O$ relative to today
- Evidence for marked changes in Antarctic hydrological cycle

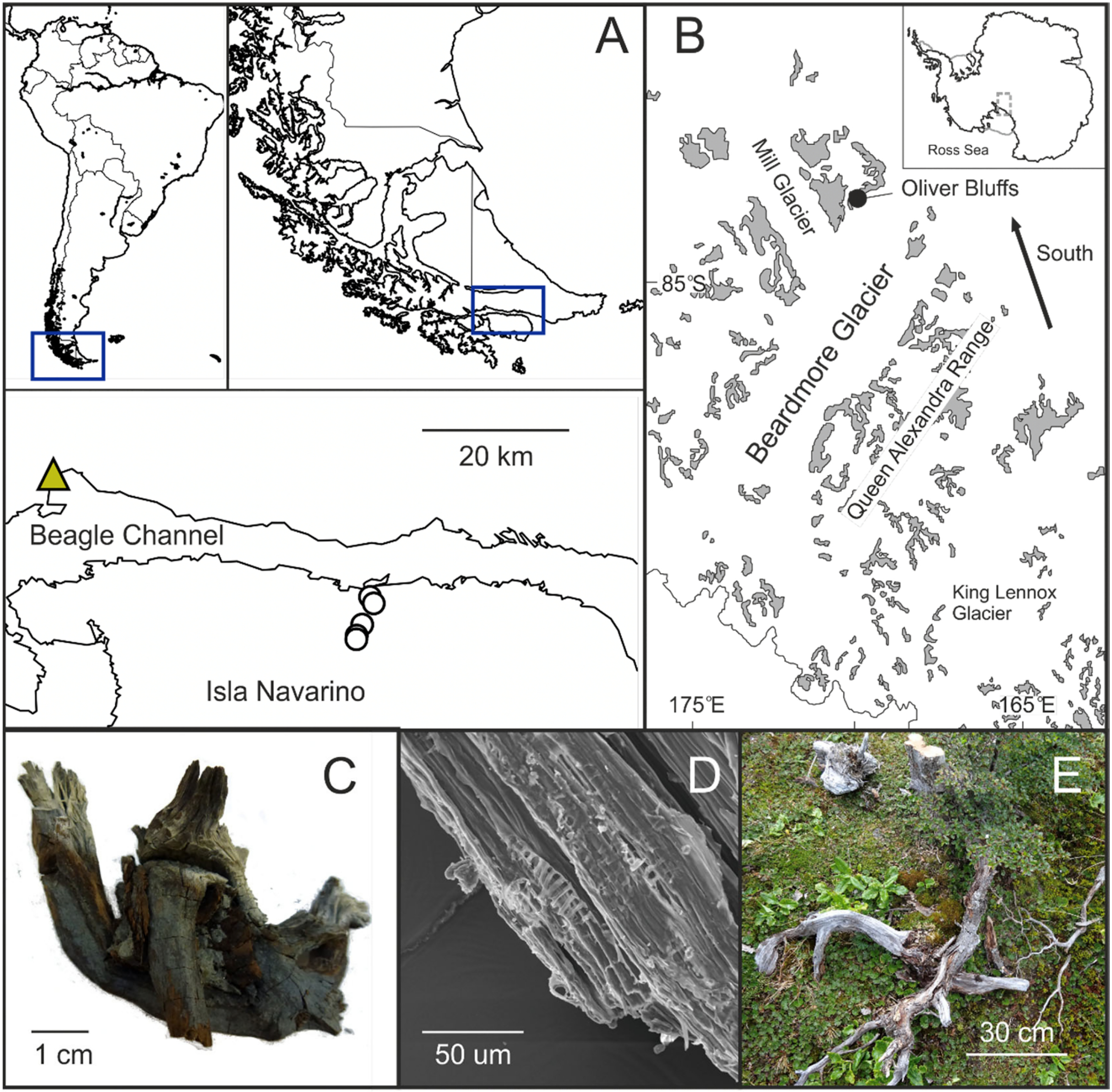

A
20 km
Beagle Channel
Isla Navarino
B
Ross Sea
Mill Glacier
Oliver Bluffs
South
85°S
Beardmore Glacier
Queen Alexandra Range
King Lennox Glacier
175°E
165°E
C
1 cm
D
50 um
E
30 cm

Figure 1

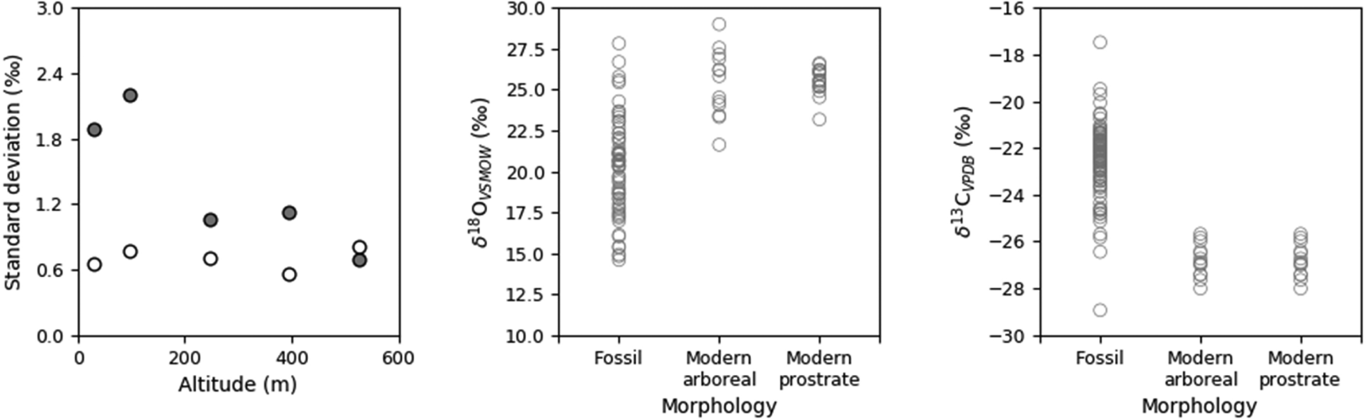

3.0
2.4
1.8
1.2
0.6
0.0
Standard deviation (‰)
0
200
400
600
Altitude (m)
30.0
27.5
25.0
22.5
20.0
17.5
15.0
12.5
10.0
$\delta^{18}O_{VSMOW}$ (‰)
Fossil
Modern arboreal
Modern prostrate
Morphology
−16
−18
−20
−22
−24
−26
−28
−30
$\delta^{13}C_{VPDB}$ (‰)
Fossil
Modern arboreal
Modern prostrate
Morphology

Figure 2

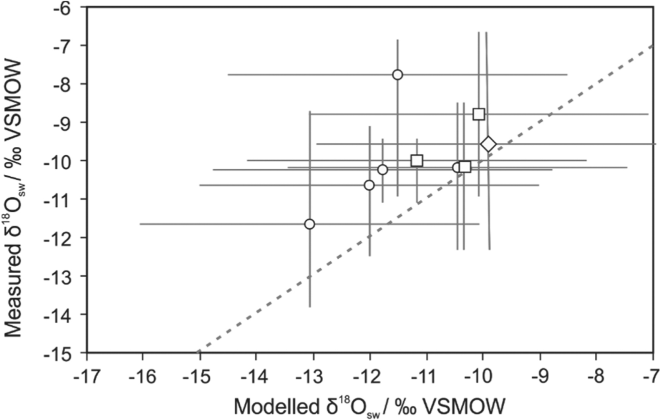
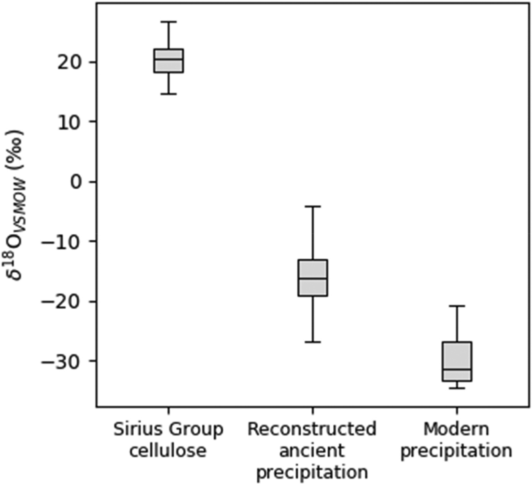

-6
-7
-8
-9
-10
-11
-12
-13
-14
-15
Measured δ18Osw / ‰ VSMOW
-17
-16
-15
-14
-13
-12
-11
-10
-9
-8
-7
Modelled δ18Osw / ‰ VSMOW

Figure 3



20
10
0
−10
−20
−30
$\delta^{18}O_{VSMOW}$ (‰)
Sirius Group cellulose
Reconstructed ancient precipitation
Modern precipitation

Figure 4