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

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HIGHLIGHTS
• Prostrate trees record average climate over decadal time periods in tree ring cellulose-δ18O.
• Late Neogene Antarctic wood indicates a ~12‰ enrichment in H2O-δ18O relative to today.
• Evidence for marked changes in Antarctic hydrological cycle

GRAPHICAL ABSTRACT

ABSTRACT
Carbon and oxygen isotopes (δ13C and δ18O) 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 δ13C and δ18O 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 δ13C 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 δ13C of contemporaneous atmospheric CO2 and enhanced water use efficiency at the fossil site. Using the cellulose-δ18O 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 δ18Oprecip 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.

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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 palaeoclimatic and environmental evolution at high temporal resolution, providing information on temperature (Gagen et al., 2007; Naulier et al., 2014; Laverge 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 et al., 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; Laverge et al., 2017b). 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 Ellisworth, 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 hydroclimatic change, for example, changes in basinal water regimes (Brien 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$_2$ (McCarl and Loader, 2004; Treydte et al., 2007); atmospheric CO$_2$ concentrations (Beierling, 1996; Köhler et al., 2010; Battaglia 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 et al., 1989; Cernusak et al., 2007; Cernusak et al., 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; Priest et al., 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 arboreal morphology are discovered 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 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 krummholz 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 et al., 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; Poud 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 seaweeds 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 et al., 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; Warmy et al., 2009; Feakins et al., 2012; Grieser et al., 2015; Gasson et al., 2016; Levy et al., 2016). Increasingly both modelling (Dolan et al., 2011; Austermann et al., 2015; Pollard et al., 2015; Pollard and Deconto, 2016) and data (Fielding et al., 2012; Cook et al., 2013; Ohnesier 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 et al., 2011; Buras and Wilming, 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 (δ¹³C and δ¹⁸O) 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 et al., 1996). Leaf remains and tree ring analyses suggest that these were deciduous prostrate shrubs, very similar to *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 fragility 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 ecosystem 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 Sirus Group sediments are deciduous (Hill et al., 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 (version 44h3; Cook and Krusic, 2014). 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 et al., 1984) was calculated for each site’s δ¹⁸O and δ¹³C 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 δ¹³C) (0.65–0.87 for δ¹⁸O; 0.46–0.76 for δ¹³C), suggesting that a greater sample size is needed to be representative of the whole sample site, particularly for δ¹³C, 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 isotope ratios 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 δ¹⁸O. 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 laboratory in the School of Earth and Environment, 2013–2016.

Oxygen isotope ratios are expressed as δ¹⁸O; where delta notation is the conventional notation used for the ratio of isotopes (e.g. δ¹⁸O/¹⁶O) in a sample (R) relative to a standard (RSTD) such that δ = ([R/RSTD] − 1) 1000 reported in per mil (‰). Results are reported with respect to Vienna Standard Mean Ocean Water (VSMOW). Carbon isotope ratios

| Site | Latitude | Longitude | Elevation/m | No. trees | δ¹⁸Omean/‰ | 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         | −10.3 ± 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                       |

Table 1

Summary of sample sites on Isla Navarino with mean site δ¹⁸O values.
(13C/12C) are expressed as δ13C 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 et al., 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 δ18O and δD, but the delignification step during extraction is performed below pH 5, which removed 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 h, 60 °C, repeated twice) to remove tannins, resins and fatty acids. Samples were then heated (100 °C) in acidified NaClO2 (via glacial acetic acid; 7.5%, pH 4–5) for 10 h; this step was repeated four times to ensure complete delignification. Finally, we used a solution of NaOH (17%; 60 °C, 2 h) to remove hemicelluloses, leaving α-cellulose for analysis. Cellulose samples were homogenised using a Retsch MM301 Mixer Mill, then freeze-dried for 24 h to remove ambient water. Samples were stored in Eppendorf vials and kept in a desiccator for >24 h prior to isotope analysis.

In order to measure δ18Osample, 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 18O/16O were converted to δ18OSMOW with a one point linear calibration using IAEA-601 (benzoic acid; δ18O = 23.15 ± 0.3‰) with reference to cellulose from Sigma-Aldrich, UK (Lot#SLBD292792; hereafter Leeds Sigma cellulose). The Leeds Sigma cellulose was analysed at the University of Leeds against IAEA-CH-3 cellulose, assuming δ18O = 31.9 ± 0.5‰ (Hunsinger et al., 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 δ13C 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 13C/12C 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-LSV (−46.479%), IAEA-CH7 (−31.83%), IAEA-CH6 (−10.45%) and IAEA-C01 (−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 et al. (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 18O/16O 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 δ18O.

The isotopic composition of plant source water for the modern *Nothofagus* in this study was constrained by measuring δ18O of soil waters (δ18Osoil) for the five sites, which ranged between −13.1 ± 0.73‰ and −10.6 ± 1.17‰ (1σ; grand mean = −11.9 ± 0.89‰; n = 16; Table 1). Oxygen isotopes from eight streams and lakes across the sampling transect (δ18Ostream), 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 δ18Ostream and δ18Oprecip, indicating that plants took up water from an annually integrated precipitation signal.

The δ18O data presented here only represent one year’s precipitation. We also used temperature, precipitation and precipitation δ18O data from the nearby Global Network of Isotopes in Precipitation (GNIP) station at Ushuaia, 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 Ushuaia was −9.9 ± 0.9‰; mean winter precipitation was −11.92 ± 0.75‰, which is not statistically different from the mean soil water δ18O (p < 0.001).

2.3.4. Modelling δ18Osource

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 δ18Osource with δ18Ocell (Rodent et al., 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).

\[
\delta^{18}O_{\text{source}} = \delta^{18}O_{\text{cell}} - (1-f)(1-RH)(\epsilon + \epsilon_k) - \epsilon
\]

(1)

where \(\epsilon\) is the biological fractionation factor associated with the formation of cellulose (+27 ± 3‰; Sternberg and DeNiro, 1983), \(\epsilon_k\) 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 (\(\epsilon_k\)) is dependent on leaf morphology and boundary layer vapour transport conditions; broad-leaf trees have quasi-laminar boundary layer conditions so \(\epsilon_k = 21‰\) (Buahy et al., 1996). The parameter \(f\) is the fraction of leaf water not subject to evaporation (Allison et al., 1985) and also includes the isotopic alteration of carbon-bound oxygen via exchange with stem water (Rodent and Ehleringer, 1999).

We tested the assumptions made by Anderson et al. (2002) using measured δ18Ocell 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...
In order to apply the model to fossil Nothofagus, we sampled between these constraints (n = 10,000) to provide an estimate of the possible range of δ18Oprecip.

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 δ18Ocell 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 δ18Ocell and therefore for altitude and morphology (i.e. prostrate or arboreal form), indicating that morphology does not impact absolute δ18Ocell 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 arboresal 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 δ13Ccell 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, δ13Ccell variability may be dominated by carbon assimilation rather than stomatal conductance (in support of findings of Farquhar et al. (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 et al., 2017a; 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 δ13Ccell was −22.6 ± 1.9‰ (1σ). 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 −14‰ (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 et al., 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 CO2 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 δ13Ccell.

Additionally, Tipple et al. (2010) find that δ13C of atmospheric CO2 was higher than present day for both the Pliocene (between around −6.7 and −6‰) and mid-Miocene (between around −5.7 and −5.5‰), compared to between −8.5 and −7.5‰ for the present day (Keeling et al., 2017). This increase in the baseline δ13C in combination with enhanced water-use efficiency, is sufficient to explain the large enrichment we see between modern and fossil δ13Ccell data.

3.3. Source water δ18O in modern Nothofagus

There was no significant trend in δ18O 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 δ18Ocell 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 $\delta^{18}O$ ($\delta^{18}O_{\text{source}}$) can be treated as $\delta^{18}O_{\text{precip}}$ where $\delta^{18}O_{\text{precip}}$ 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 $\delta^{18}O_{\text{precip}}$ can be reconstructed from $\delta^{18}O_{\text{cell}}$ using the model from Anderson et al. (2002).

The chosen model under-predicted $\delta^{18}O_{\text{source}}$ by between 0.2‰ and 2.9‰ (mean for all sites = 1.5‰; Fig. 3). This could be due to model parameterization; we chose a value of exactly 27% for the biological fractionation factor $\varepsilon$, 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 $\delta^{18}O_{\text{source}}$ was not statistically different from $\delta^{18}O_{\text{root}}$, $\delta^{18}O_{\text{stream}}$, or GNIP summer precipitation, indicating that the model works well for predicting $\delta^{18}O_{\text{precip}}$ from measured $\delta^{18}O_{\text{cell}}$ in Nothofagus. We now apply the model to the fossil Nothofagus in order to calculate ancient $\delta^{18}O_{\text{precip}}$.

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

Mean $\delta^{18}O_{\text{cell}}$ 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 100kyr, 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 $\delta^{18}O_{\text{cell}}$ 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 $\delta^{18}O_{\text{cell}}$, 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 $\delta^{18}O_{\text{cell}}$ could be caused by a difference in $\delta^{18}O_{\text{precip}}$, 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 $\delta^{18}O_{\text{precip}}$ from Anderson et al. (2002). Using this approach, we calculate that mean continental Antarctic palaeoprecipitation was $-16 ± 4.2‰$ (1σ; range between −26‰ and –3.5‰). Since $\delta^{18}O_{\text{cell}}$ 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, $\delta^{18}O_{\text{precip}}$ 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 $\delta^{18}O_{\text{precip}}$ values. We therefore compared our record to measured Antarctic $\delta^{18}O_{\text{precip}}$ 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 $\delta^{18}O_{\text{precip}}$ was significantly enriched by $-11.2‰$ relative to modern $\delta^{18}O_{\text{precip}}$ (ancient mean = $-16‰$; modern mean = $-28‰$; $p < 0.001$; Fig. 4). Growth experiments have suggested that plant $\delta^{18}O$ (and therefore by extension, $\delta^{18}O$) 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 $\delta^{18}O_{\text{cell}}$ 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 et al., 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 contemporary terrestrial temperature proxies, which suggest that summer temperatures reached 5°C during the period of study.

**Fig. 3.** The relationship between modelled source water $\delta^{18}O$ and measured $\delta^{18}O_{\text{source}}$ 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 $\delta^{18}O_{\text{precip}}$ (summer precipitation; diamond). Modelled source water $\delta^{18}O$ was calculated from measured $\delta^{18}O_{\text{cell}}$ (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 $\delta^{18}O_{\text{source}}$ if $\varepsilon$ was 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, $f = 0.2$.  

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degrees warmer than today (Warny et al., 2009; McKay et al., 2012; with both age scenarios for the site: during both the mid-Miocene and present-day). Suggests that higher and precipitation isotopes earlier in the Miocene (20 My) imply that the relationship between temperature and precipitation isotopes correlates more strongly with contemporary atmospheric 

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.

Fig. 4. Cellulose δ\(^{18}\)O from the Sirius Group fossil Nothofagus, with modelled δ\(^{18}\)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.

Table 1. Summary of tree ring stable isotope studies in both palaeo and modern times for tree ring isotope analysis. This result opens up high latitude and altitude end-member conditions which 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 δ\(^{18}\)O \textsubscript{cell} with δ\(^{18}\)O \textsubscript{precip} in two species of modern Nothofagus plants, which grow in both arboreal and prostrate form, we found that δ\(^{18}\)O \textsubscript{cell} of prostrate Nothofagus faithfully records δ\(^{18}\)O \textsubscript{precip} at multi-year resolution. However, 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 \textit{krummholz}-type plants, such as those from Oliver Bluffs, which feature growth symmetry 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 conditions in both palaeo and modern times for tree ring isotope analysis.

CRediT authorship contribution statement

Rhian L. Rees-Owen: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Visualization. Rob J. 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 competing interest

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142247.
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