UNIVERSITY OF LEEDS

This is a repository copy of *A calibration of cellulose isotopes in modern prostrate Nothofagus and its application to fossil material from Antarctica*.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/165439/

Version: Accepted Version

White Rose
university consortium
Universities of Leeds, Sheffield & York

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

1   A calibration of cellulose isotopes in modern prostrate *Nothofagus* and its

2   application to fossil material from Antarctica

3   Rhian L. Rees-Owen[a,1], Rob J. Newton[a,*], Ruza F. Ivanovic[a], Jane E. Francis[b], James B.

4   Riding[c], Alina D. Marca[d]

5   [a]*School of Earth and Environment, University of Leeds, Leeds, UK*

6   [b]*British Antarctic Survey, Cambridge, UK*

7   [c]*British Geological Survey, Nottingham, UK*

8   [d]*School of Environmental Sciences, University of East Anglia, Norwich, UK*

9   [1]*Present Address: Department for Business, Energy, and Industrial Strategy, 1 Victoria St, Westminster,*

10   *London SW1H 0ET*

11   *corresponding author: r.j.newton@leeds.ac.uk

12   **Abstract**

13   Carbon and oxygen isotopes ($\delta^{13}C$ and $\delta^{18}O$) in tree rings are widely used to reconstruct

14   palaeoclimate variables such as temperature during the Holocene (12 thousand years ago -

15   present), and are used increasingly in deeper time. However, their use is largely restricted to

16   arboreal trees, which excludes potentially important data from prostrate trees and shrubs, which

17   grow in high latitude and altitude end-member environments. Here, we calibrate the use of $\delta^{13}C$

18   and $\delta^{18}O$ as climatic archives in two modern species of southern beech (*Nothofagus*) from

19   Tierra del Fuego, Chile, at the southern limit of their current range. We show that prostrate trees

20   are potentially suitable archives for recording climatological means over longer periods (on the

21   order of decades), which opens up these important environments for tree ring isotope analysis.

22   We then apply our new understanding to a remarkable late Neogene (17-2.5 Ma) fossil

23   *Nothofagus* assemblage from the Transantarctic Mountains, Antarctica, representative of a

24   prostrate tundra shrub growing during a period of significant ice sheet retreat. The $\delta^{13}C$ of the

25   fossil cellulose was found to be ~4‰ enriched relative to that of the modern tress. This is likely

26   to be due to a combination of a more positive $\delta^{13}C$ of contemporaneous atmospheric CO_2 and

27   enhanced water use efficiency at the fossil site. Using the cellulose-$\delta^{18}O$ in the fossil wood, we

28   are able to reconstruct precipitation oxygen isotopes over the Antarctic interior for the first time

29   for this time period. The results show that $\delta^{18}O_{precip}$ over Antarctica was -16.0±4.2‰, around

30   12‰ enriched relative to today, suggesting changes in the hydrological cycle linked to warmer

31   temperatures and a smaller ice sheet.

32

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

34   **1   Introduction**

35   Tree ring stable isotope analysis is a powerful and widely-used tool for palaeo-climatic

36   reconstructions (Cernusak and English, 2014; Gessler *et al.*, 2014). It can provide rare insights

37   into terrestrial palaeo-climate and environmental evolution at high temporal resolution, providing

38   information on temperature (Gagen *et al.*, 2007; Naulier *et al.*, 2014; Lavergne *et al.*, 2016,

39   2018), precipitation (Cullen and Grierson, 2009; Xu *et al.*, 2016), drought (Kress *et al.*, 2010;

40   Labuhn *et al.*, 2016), and large-scale atmospheric circulation patterns (Xu, Sano and

41   Nakatsuka, 2013; Grießinger *et al.*, 2018).

42   A key source of information in much of this work is the oxygen isotopic composition of tree ring

43   cellulose ($\delta^{18}O_{cell}$). The theory on the underpinning variables controlling $\delta^{18}O_{cell}$ is relatively well

44   developed, albeit with large uncertainties and knowledge gaps e.g. (Gessler *et al.*, 2014;

45   Treydte *et al.*, 2014). The relationship between these variables and $\delta^{18}O_{cell}$ can be described by

46   various numerical models and used to investigate oxygen isotope variations in multiple settings

47   (Roden and Ehleringer, 2000; Farquhar and Gan, 2003; Ogée *et al.*, 2003, 2009; Danis *et al.*,

48   2012; Lavergne, Gennaretti, *et al.*, 2017). Cellulose oxygen isotopes are governed by a complex

49   array of factors, including source water isotopic composition (itself a result of precipitation

50   isotopes, soil residence time, and evaporative effects); leaf water enrichment due to

51   transpiration (Yakir and Sternberg, 2000); fractionation between leaf water and carbonyl oxygen

52   (Sternberg and DeNiro, 1983; Sternberg and Ellsworth, 2011); and other oxygen exchange

53   processes between organic compounds and surrounding water, for example during

54   remobilisation of organic matter or cellulose biosynthesis (e.g. Hill *et al.*, 1995; Sternberg *et al.*,

55   2006; Gessler *et al.*, 2007; Offerman *et al.*, 2011; Nabeshima *et al.*, 2018). The underpinning

56   link with source water oxygen isotopes means that tree-ring cellulose $\delta^{18}O$ can be used to

57   reconstruct the oxygen isotopic composition of precipitation. This in itself is a function of

58   precipitation amount, altitude, temperature, residence time in the atmosphere, distance from

59   moisture source and transport patterns (Dansgaard, 1964; Sime *et al.*, 2009; Aggarwal *et al.*,

60   2012). Cellulose $\delta^{18}O$ can therefore be used as a proxy for reconstructing global and regional

61   hydrological change, for example, changes in basinal water regimes (Brienen *et al.*, 2012) or

62   large-scale atmospheric circulation patterns (Baldini *et al.*, 2008; Zhu *et al.*, 2012).

63   Carbon isotopes in tree-ring cellulose ($\delta^{13}C_{cell}$) also have utility as a palaeoclimatic proxy. In

64   general, $\delta^{13}C_{cell}$ is controlled by the $\delta^{13}C$ of atmospheric CO_2 (McCarroll and Loader, 2004;

65   Treydte *et al.*, 2007); atmospheric CO_2 concentrations (Beerling, 1996; Köhler *et al.*, 2010;

66   Battipaglia *et al.*, 2013), along with other factors that affect stomatal conductance including soil

67   moisture and atmospheric vapour pressure deficit; and factors that control photosynthetic

68   capacity such as nutrient availability and irradiance (Ehleringer *et al.*, 1986; Farquhar,

69   Ehleringer and Hubick, 1989; Cernusak *et al.*, 2007; Cernusak, Winter and Turner, 2009). A

70   range of downstream metabolic processes also play a role in shaping $\delta^{13}C_{cell}$, including post-

71   carboxylation fractionation, phloem loading and transport, and respiratory isotope fractionation

72   (Gessler *et al.*, 2009; Priault, Wegener and Werner, 2009; Werner and Gessler, 2011; Werner *et*

73   *al.*, 2011).

74   Both carbon and oxygen tree ring isotopes are increasingly being applied to older time periods

75   of up to 53 Ma as more fossil plants with adequate preservation are being recovered (Jahren

76   and Sternberg, 2008; Schubert and Jahren, 2011; Schubert *et al.*, 2012; Wolfe *et al.*, 2012;

77   Hare *et al.*, 2018). One particular advantage of this growing dataset is the ability of tree ring

78   isotopes to reconstruct climatic parameters that are much harder to access through marine

79   sediments. These include environmental geochemical signals like precipitation isotopes

80   (Ballantyne *et al.*, 2006; Jahren and Sternberg, 2008; Jahren *et al.*, 2009) and atmospheric

81   carbon isotopes (Arens, Jahren and Amundson, 2000; Jahren *et al.*, 2001). A notable example

82   is the rich treasure trove of exceptionally well-preserved Eocene and Pliocene fossil wood from

83   multiple kimberlite deposits in the Canadian High Arctic. These fossil recoveries have revealed

84   unique details about Eocene and Pliocene palaeoclimate and hydrological cycling through their

85   stable isotope records, such as reconstructing terrestrial temperatures and the isotopic

86   composition of precipitation as well as providing insights into high latitude climate variability

87   (Ballantyne *et al.*, 2006, 2010; Jahren and Sternberg, 2008; Jahren *et al.*, 2009; Csank *et al.*,

88   2011; Wolfe *et al.*, 2012).

89   In this study, we apply tree ring isotope analysis to a unique suite of fossil prostrate or

90   *krummoltz Nothofagus* trees recovered from the mid-late Neogene (~17 – 2.5 Ma) Sirius Group

91   deposits at the Oliver Bluffs in the Transantarctic Mountains, Antarctica (85°07'S, 166°35'E;

92   Webb and Harwood, 1987, 1993; Francis and Hill, 1996; Hill, Harwood and Webb, 1996). The

93   plants were deposited at a similar latitude to today (Lawver and Gahagan, 2003) and represent

94   a period of significant Antarctic Ice Sheet retreat, where warming of the continent allowed a

95   tundra-like shrub to grow 480 km from the South Pole.

96   Based on both geochemical (Rees-Owen *et al.*, 2018) and macrofossil-derived (Francis and Hill,

4

97 1996; Ashworth and Cantrill, 2004) palaeothermometers, continental summer temperatures

98 during the trees' lifetimes were ~5°C, implying a weakened latitudinal temperature gradient

99 compared to the present day, where the mean temperature in December is -3.4°C (McMurdo

100 Station; 77°51'S, 166°40'E). Shallower gradients are also supported by vegetation and marine

101 proxy-based reconstructions, indicating, for example, a reduction of ~5.5°C in the meridional

102 temperature gradient during the early Pliocene relative to today (Brierley *et al.*, 2009; Pound *et*

103 *al.*, 2012).

104 The age of these sediments has been the subject of a lengthy debate, relating to the nature of

105 the East Antarctic Ice Sheet under warmer-than-present conditions (Barrett, 2013).

106 Biostratigraphical dating of the plant fossils by association with late Pliocene marine diatoms

107 (Webb *et al.*, 1984; Harwood, 1986) suggests the incursion of seaways deep into the Antarctic

108 interior and indicates a dynamic ice sheet as late as 3 million years ago. This relatively young

109 age for the plant fossils has been challenged by suggestions that the diatoms represent wind-

110 blown contamination from the open ocean (Burckle and Potter, 1996; Stroeven, Prentice and

111 Kleman, 1996). Furthermore, cosmogenic exposure dating of nearby moraines indicates these

112 sediments are much older (at least 5 Ma, but possibly as old as 17 Ma; Ackert, Jr. and Kurz,

113 2004) and therefore that the ice sheet has been a stable climatic feature since the mid-Miocene.

114 Evidence for a periodically reduced ice sheet accompanied by vegetation along the margins

115 exists for the mid-Miocene (17-15 Ma; Warny *et al.*, 2009; Feakins, Warny and Lee, 2012;

116 Griener *et al.*, 2015; Gasson *et al.*, 2016; Levy *et al.*, 2016). Increasingly both modelling (Dolan

117 *et al.*, 2011; Austermann *et al.*, 2015; Pollard, Deconto and Alley, 2015; Pollard and Deconto,

118 2016) and data (Fielding *et al.*, 2012; Cook *et al.*, 2013; Ohneiser *et al.*, 2020) studies also

119 suggest that at least partial EAIS retreat occurred during the Pliocene, allowing a tundra shrub

120 to grow around 4.1 Ma. These competing scenarios pose a challenge to dating these fossils.

121 Nevertheless, the fossiliferous bed clearly represents a period of significant East Antarctic Ice

122   Sheet (EAIS) retreat in response to warming temperatures (Mercer, 1986; Francis and Hill,

123   1996). Our data will therefore give novel insight into past Antarctic climate change during a vital

124   period in its glacial history.

125   To date, the vast majority of tree ring stable isotope studies have been applied to trees with an

126   arboreal habit. Prostrate trees (where stems grow horizontally to avoid harsh conditions such as

127   freezing winds e.g. *Salix arctica* in the High Arctic and the fossil plants considered in this study)

128   and shrubs are increasingly used in modern dendrochronological studies (Woodcock and

129   Bradley, 1994; Hantemirov, Shiyatov and Gorlanova, 2011; Buras and Wilmking, 2014), where

130   they can provide vital information on past climate for tree-less regions such as those at high

131   latitude or altitude, and deserts. However, to our knowledge, no studies using tree ring isotopes

132   in prostrate plants to reconstruct past climate exist, so there is uncertainty over the extent to

133   which isotope theory developed for arboreal tree rings holds true for *krummholz*-type plants.

134   Therefore, the objective of the first part of this study is to calibrate the use of tree ring isotopes

135   (δ^{13}C and δ^{18}O) in high latitude prostrate trees for climatic reconstructions using plants from Isla

136   Navarino, Chile, where two deciduous southern beech (*Nothofagus*) species grow in both

137   arborescent and prostrate form in a subpolar forest environment at the southern limit of their

138   range. The objective of the second part is to apply this new knowledge to our fossil *Nothofagus*

139   trees to enhance our understanding of how the Antarctic Ice Sheet has behaved during past

140   warm periods of Earth's history.

141   **2   Materials and methods**

142   *2.1   Oliver Bluffs; fossil site*

143   The fossil wood was sampled from a sedimentary succession at Oliver Bluffs in the Dominion

144   Range of the Transantarctic Mountains (85°07'S, 166°35'E), which forms part of the Sirius

145   Group sediments (Fig. 1). The fossil plant material occurs within one main bedding horizon in

146   the central part of the exposure at Oliver Bluffs, on the eastern side of the upper valley of the

6

147   Beardmore Glacier. The present elevation is approximately 1760 m above sea level, but

148   deposition likely occurred at a much lower altitude (Webb and Harwood, 1993; Ackert, Jr. and

149   Kurz, 2004). The sedimentary sequence consists of glacial diamictites, and are thought to be

150   lodgement tills deposited by the ancestral Beardmore Glacier during glacial advance and retreat

151   (McKelvey *et al.*, 1991). The fossiliferous bed containing fossil wood and leaves comprises

152   poorly-sorted sandstones with silt lenses, representing an outwash deposit, in places burying

153   poorly-developed glacial soils on a braided outwash plain (Ashworth and Cantrill, 2004). We

154   envisage sporadic accretion of sediment over the plain, such that the fossiliferous bed is

155   spatially heterogeneous, but as a whole is representative of a significant portion of the ice sheet

156   retreat event (Rees-Owen *et al.*, 2018).

157   The fossil wood fragments were first described as *Nothofagus* (Carlquist, 1987) and later

158   identified as *Nothofagus beardmorensis* and are dated to between 17 and 2.5 Ma (Hill and

159   Jordan, 1996; Hill, Harwood and Webb, 1996). Leaf remains and tree ring analyses suggest that

160   these were deciduous prostrate shrubs, very similar to the *krummholz N. pumilio* and *N.*

161   *antarctica*, which grow at the treeline in Tierra del Fuego, Chile (Francis and Hill, 1996). Due to

162   the small ring size (<100 µm) and friability of the material, fossil wood fragments were sampled

163   for isotope analysis in bulk or by isolating individual rings where possible, so our measurements

164   are averages over multiple years and up to several decades. The necessity of combining

165   multiple rings together for the analysis of the fossil wood material sets the context for the

166   modern part of our study in which we stress the interpretation of data on decadal rather than

167   annual timescales.

168   *2.2   Isla Navarino; modern analogue site*

169   Isla Navarino (55°56'S, 67°37'W; Fig. 1) is part of the Magellanic subpolar forests ecoregion

170   which stretches west of the Andes down to Tierra del Fuego, Chile. The island has a maritime

171   climate, with mean annual temperatures of 6 °C, average summer highs of 10°C and winter

172 averages of 2°C. Cool windy conditions prevail year round; Mean Annual Precipitation (MAP) is

173 400-500 mm, which is uniformly distributed throughout the year. The island vegetation is

174 characterised by Magellanic forest dominated by *Nothofagus* trees to the north, and Magellanic

175 moorland to the south.

176 The overall intention of this study is to ascertain whether the Antarctic fossil trees can be used

177 for tree ring isotope work, so we designed our sampling strategy for the modern plants to mimic

178 this where possible, including limiting our study to *Nothofagus* only. Wood cores and rounds

179 from branches (for prostrate trees) from 31 living trees were collected at five sites on Isla

180 Navarino during the austral summer of 2013. Three species of *Nothofagus* trees grow on the

181 island, one evergreen species (*N. betuloides*) and two deciduous species (*N. antarctica* and *N.

182 pumilio*). Because the fossil *Nothofagus* from the Sirus Group sediments are deciduous (Hill,

183 Harwood and Webb, 1996), cores were taken from two deciduous *Nothofagus* species over an

184 altitude transect from near sea-level to the treeline (~600 m) at 5 sites (Table 1; Fig. 1). Over

185 the transect, *Nothofagus* ranged in habit from arborescent (single stem and generally greater

186 than 4 m in height) to *krummholz* form (i.e. prostate, with a small trunk or stem and multiple

187 branches lying horizontally upon the ground). Species were identified by leaf character (Moore,

188 1983) and sampled during the height of austral summer 2013 (January), when the trees were in

189 full leaf. Arborescent trees were cored at chest height (~130 cm above the ground) using an

190 increment wood corer with a diameter of 5 mm. Prostrate individuals were sampled from primary

191 branches in order to match sampling from the fossil trees. Two cores or rounds were sampled

192 per individual tree and the cores and rounds were air-dried; cores were stored in plastic straws.

193 Rounds were sanded with progressively fine sandpaper, and the surface of the tree-ring cores

194 were cut using a core-microtome to improve ring visibility.

195 The core samples were dated to the calendar year of their formation and cross-dated using the

196 techniques described in (Stokes and Smiley, 1968). These were then statistically tested using

197   the programme COFECHA (Holmes, 1983; Grissino-Mayer, 2001) and chronologies were

198   constructed using ARSTAN. As the austral growing season overlaps two calendar years, rings

199   were assigned to the year when ring growth began (i.e. the last complete ring taken for each

200   core in January 2013 was dated to austral summer 2011, as the 2012-2013 ring was still

201   incomplete at the time of sampling).

202   A 30 year sequence was isolated for isotopic analysis covering the period 1981-2011. This

203   sequence length was chosen to roughly match the available tree ring spans of the fossil trees

204   sampled here. Tree rings are composed of earlywood and latewood; the former comprises large

205   thin-walled cells made of stored photosynthates from the previous year and the latter comprises

206   thicker-walled cells formed during summer. Therefore to sample at true annual resolution, it has

207   been suggested that only latewood should be taken (Switsur et $al.$, 1995). However, the rings in

208   the prostrate plants in this study were too small to obtain sufficient latewood, so the entire ring

209   was sampled each time; this approach has been used successfully to reconstruct temperature

210   in the same region (Lavergne et $al.$, 2016). Isotope ratios were measured separately for each

211   year and each tree. There are multiple missing years in the isotope chronologies where rings

212   were too small to extract sufficient cellulose for analysis.

213   Chronologies at annual resolution require the construction of chronologies that are statistically

214   representative of the variability of the site. An Expressed Population Signal (EPS; Wigley, Briffa

215   and Jones, 1984) was calculated for each site's $\delta^{18}O$ and $\delta^{13}C$ chronologies. This is a measure

216   of how well a chronology constructed from a finite number of trees represents the hypothetical

217   perfect or true chronology; a value of 0.85 is generally considered to be an acceptable

218   confidence level. On the whole, EPS is highly sensitive to the number of trees in the chronology.

219   In this study, the EPS for each site was low (particularly for $\delta^{13}C$) (0.65 – 0.87 for $\delta^{18}O$; 0.46 –

220   0.76 for $\delta^{13}C$), suggesting that a greater sample size is needed to be representative of the whole

221   sample site, particularly for $\delta^{13}C$, which generally exhibits lower EPS (Daux et $al.$, 2018).

222 Because we are not intending to develop a detailed chronology for Isla Navarino, but instead

223 test whether tree ring isotopes are broadly applicable to our prostrate fossil trees, we judge that

224 this is adequate for the purposes of this study.

225

226 Soil and root samples were also collected, along with water from a stream network covering the

227 altitude transect in order to estimate source water $\delta^{18}O$. Soils were sampled from 50 cm depth

228 around the roots of three trees from each of the five sites (where 90% of *Nothofagus* forest root

229 mass is situated; Schulze *et al.,* 1996). Root samples were taken from at least one tree at three

230 of the five sites. Roots and soils were wrapped in cling film, stored in multiple airtight bags and

231 frozen until required for water extraction. Source water samples were taken from seven fast-

232 flowing streams and one lake, covering the entire altitudinal transect, filtered (0.2 µm), and

233 stored in McCartney vials.

234 *2.3   Sample preparation and isotopic analysis*

235 Except where otherwise indicated, the following procedures were all carried out in the University

236 of Leeds Cohen Geochemistry laboratories in the School of Earth and Environment, 2013 -

237 2016.

238 Oxygen isotope ratios are expressed as $\delta^{18}O$; where delta notation is the conventional notation

239 used for the ratio of isotopes (e.g. $^{18}O/^{16}O$) in a sample (R) relative to a standard (R_{STD}) such

240 that $\delta = (R/(R_{STD} - 1)1000,)$ reported in per mil (‰). Results are reported with respect to Vienna

241 Standard Mean Ocean Water (VSMOW). Carbon isotope ratios ($^{13}C/^{12}C$) are expressed as $\delta^{13}C$

242 and reported relative to the Vienna Pee Dee Belemnite standard.

243

244 *2.3.1   Preservation of fossil material*

245 Exceptional preservation of the fossil *Nothofagus* utilised in this study is well documented

246 (Francis and Hill, 1996), and is supported by scanning electron microscope imaging (Fig 1D),

247 which shows excellent retention of wood fibres. Although it is clear that some degradation of

248 vessels has occurred, this should not impact the isotopic signal of the remaining cellulose;

249 cellulose extracted from fossil trees significantly older than those used in this study (up to 53

250 Ma; (Wolfe *et al.*, 2012; Hook *et al.*, 2014, 2015; Staccioli, Santoni and Pizzo, 2014) was

251 extracted in low yield (<5%; Hook *et al.*, 2015) indicating a high degree of cellulose degradation,

252 but showed no signs of isotopic alteration. Mineral contaminants in the form of microcrystalline

253 calcite were detected in the Sirius Group fossil trees using energy dispersive X-ray

254 spectroscopy, which could affect both $\delta^{18}O$ and $\delta^{13}C$, but the delignification step during

255 extraction is performed below pH 5, which removed all calcite. After extraction, cellulose was

256 recovered as a white fluffy material (5 - 30% yield), which is a clear indication that cellulose is

257 well-preserved and hence the fossil material is appropriate for isotope analysis.

258 *2.3.2   Cellulose isotope measurements*

259 Cellulose was extracted from both modern and fossil samples using batch extraction equipment

260 described by (Wieloch *et al.*, 2011). To summarise, ground wood samples were heated in

261 aqueous NaOH solution (5%, 2 hours, 60°C, repeated twice) to remove tannins, resins and fatty

262 acids. Samples were then heated (60°C) in acidified $NaClO_2$ (via glacial acetic acid; 7.5%, pH 4-

263 5) for 10 hours; this step was repeated four times to ensure complete delignification. Finally, we

264 used a solution of NaOH (17%; 60°C, 2 hours) to remove hemicelluloses, leaving α-cellulose for

265 analysis. Cellulose samples were homogenised using a Retsch MM301 Mixer Mill, then freeze-

266 dried for 24 hours to remove ambient water. Samples were stored in Eppendorf vials and kept in

267 a desiccator for >24 hours prior to isotope analysis.

268   In order to measure $\delta^{18}O_{cell}$, the milled, freeze-dried cellulose samples were weighed, packed

269   into silver capsules and pyrolysed at 1450°C. Oxygen isotope ratios were measured using an

270   elemental analyser with a purge and trap column (Elementar vario PYRO cube), coupled to an

271   Isoprime isotope ratio-mass spectrometer. Ratios of $^{18}O/^{16}O$ were converted to $\delta^{18}O_{VSMOW}$ with a

272   one point linear calibration using IAEA-601 (benzoic acid; $\delta^{18}O$ = 23.15±0.3‰) with reference to

273   cellulose from Sigma-Aldrich, UK (Lot#SLBD2972V; hereafter Leeds Sigma cellulose). The

274   Leeds Sigma cellulose was analysed at the University of Leeds against IAEA-CH-3 cellulose,

275   assuming $\delta^{18}O$ = 31.9±0.5‰ (Hunsinger, Hagopian and Jahren, 2010) and assigned a value of

276   29.2±0.2 . Standards were included at an interval of every twelve samples. Within-run

277   reproducibility of an internal check standard was ±0.37‰. For $\delta^{13}C$ analysis, extracted cellulose

278   samples were weighed and packed into tin capsules. Carbon isotope ratios were measured

279   using an Elementar vario PYRO cube elemental analyser coupled to an Isoprime mass

280   spectrometer. The encapsulated samples were combusted at 1150°C in pure oxygen. Ratios of

281   $^{13}C/^{12}C$ were calibrated to the international VPDB scale using in-house urea and C4 sucrose.

282   These were assigned values of -46.83±0.22‰ and -11.93±0.24‰, respectively after calibration

283   using six replicates of each of the following international standards: IAEA-LSVEC (-46.479‰),

284   IAEA-CH7 (-31.83‰), IAEA-CH6 (-10.45‰) and IAEA-CO1 (+2.48‰). The precision obtained

285   for repeat analysis was better than ±0.2‰ (σ).

286

287   *2.3.3   Water isotope measurements*

288   Water was extracted from roots and soils by cryogenic vacuum distillation, following the

289   procedure detailed by West, Patrickson and Ehleringer (2006). Extracted samples, along with

290   stream waters, were stored frozen until they were measured for water isotope ratios at the

291   School of Environmental Sciences, University of East Anglia, UK. The $^{18}O/^{16}O$ ratios were

292 analysed using a Picarro L1102-i cavity ring-down spectroscopy analyser with a CTC Analytics

293 autosampler. Each sample was injected and measured 6 times using 2.5 µl of water for each

294 injection. Together with the samples, two secondary international standards (USGS 64444 and

295 USGS 67400) and one internal laboratory standard (NTW – Norwich tap water) were measured,

296 each injected 10 times in order to minimize memory effects. Final isotopic compositions were

297 calculated using the calibration line based on the secondary international standards and

298 reported in permil units with respect to V-SMOW on the V-SMOW – SLAP scale. The precision

299 of the measurements is 0.1 ‰ for $\delta^{18}O$.

300 The isotopic composition of plant source water for the modern *Nothofagus* in this study was

301 constrained by measuring $\delta^{18}O$ of soil waters ($\delta^{18}O_{soil}$) for the five sites, which ranged between -

302 13.1±0.73‰ and -10.6±1.17‰ (1 σ; grand mean = -11.9±0.89‰; n=16; Table 1). Oxygen

303 isotopes from eight streams and lakes across the sampling transect ($\delta^{18}O_{stream}$), ranged between

304 -11.1‰ and -9.8‰ (mean = -10.8±0.41‰). Root water extracted from *Nothofagus* trees at three

305 sites (mean = -10.5±0.54‰, n=4) was isotopically similar to $\delta^{18}O_{stream}$ and $\delta^{18}O_{precip}$, indicating

306 that plants took up water from an annually integrated precipitation signal.

307 The $\delta^{18}O$ data presented here only represent one year's precipitation. We also used

308 temperature, precipitation and precipitation $\delta^{18}O$ data from the nearby Global Network of

309 Isotopes in Precipitation (GNIP) station at Ushaia, Argentina (54°46'48" S; 68°16'48" W),

310 approximately 50 km away, in order to take into consideration summer and winter seasonal

311 precipitation in this study, noting that there are a number of missing years for the data set; a

312 more complete dataset is available from Punta Arenas but this station is significantly further

313 away. Mean summer precipitation for Ushaia was -9.9±0.9‰; mean winter precipitation was -

314 11.92±0.75‰, which is not statistically different from the mean soil water $\delta^{18}O$ (p<0.001).

315 *2.3.4 Modelling $\delta^{18}O_{source}$*

316 There are multiple models of varying complexity linking these parameters and it is not clear

317 whether more complex models provide better predictions than simpler ones. For the purposes of

318 this study, we used a relatively simple model given by eq. 1 (Anderson *et al.*, 2002), which was

319 chosen because there are only two unconstrained parameters (relative humidity, RH, and the

320 fraction of leaf water not subject to fractionation, *f*). We acknowledge that there are more

321 complex process-based and mechanistic models described in the literature, but consider that

322 the use of more complex models linking $\delta^{18}O_{source}$ with $\delta^{18}O_{cell}$ (Roden, Lin and Ehleringer, 2000;

323 Danis *et al.*, 2012) would require making assumptions about a larger number of parameters

324 which are difficult to constrain in deep time, for example amount of precipitation and daily max

325 and min temperatures. The Anderson model has been used in multiple studies to reconstruct

326 past precipitation isotopes (Csank *et al.*, 2011; Wolfe *et al.*, 2012; Hook *et al.*, 2015).

327 $\delta^{18}O_{source} = \delta^{18}O_{cell} - (1 - f)(1 - RH)(\varepsilon_e + \varepsilon_k) - \varepsilon$ (1)

328 where ε is the biological fractionation factor associated with the formation of cellulose (+27±3‰;

329 (Sternberg and DeNiro, 1983), ε_e is the equilibrium liquid-vapour fractionation for water and

330 approximates $\delta^{18}O$ of atmospheric vapour (assumed here to be 11‰; Majoube, 1971) and the

331 subscript *source* denotes source water. The kinetic liquid-vapour fractionation (ε_k) is dependent

332 on leaf morphology and boundary layer vapour transport conditions; broad-leaf trees have

333 quasi-laminar boundary layer conditions so $\varepsilon_k = 21‰$ (Buhay, Edwards and Aravena, 1996).

334 The parameter *f* is the fraction of leaf water not subject to evaporation (Allison, Gat and Leaney,

335 1985) and also includes the isotopic alteration of carbon-bound oxygen via exchange with stem

336 water (Roden and Ehleringer, 1999).

337 We tested the assumptions made by Anderson et al. (2002) using measured $\delta^{18}O_{cell}$ from the

338 modern analogue trees as input for the model (with RH = 0.7, *f* = 0.2 as in Allison et al., 1985)

339   and compared the results against measured $\delta^{18}O_{source}$ (i.e. soil and stream water) $\delta^{18}O_{root}$ from

340   Isla Navarino, and the GNIP precipitation data from Ushaia. In order to apply the model to fossil

341   *Nothofagus*, we applied a large range of values for RH that are consistent with measurements

342   from high latitude modern analogue sites such as Isla Navarino (0.5 - 0.85) and using a random

343   number generator with uniform distribution, we sampled between these constraints (n=10000) to

344   provide an estimate of the possible range of $\delta^{18}O_{precip}$.

345   **3. Results and discussion**

346   *3.1. Oxygen and carbon isotope ratios in modern Nothofagus*

347   Oxygen and carbon isotope ratios in modern *Nothofagus* trees over a range of morphologies

348   were measured to provide a first order check on the ability of fossil prostrate *Nothofagus* plants

349   to record long-term climate and environmental variables. Mean $\delta^{18}O_{cell}$ for all sites over the

350   sample period (1981-2011) ranged between 24.1‰ and 26.9‰. There was no statistically

351   significant difference between the two Nothofagus species (p>0.7; Student's unpaired *t*-test).

352   However, there is also no statistically significant difference between sites for mean $\delta^{18}O_{cell}$, and

353   therefore for altitude and morphology (i.e. prostrate or arboreal form), indicating that morphology

354   does not impact absolute $\delta^{18}O_{cell}$ integrated over multiple tree rings. Intriguingly, prostrate trees

355   in this study exhibit much lower inter-tree variability than their arborescent counterparts (σ =

356   2.1‰ and σ = 0.8‰, for arboreal and prostrate morphologies, respectively (Fig. 3)). Prostrate

357   plants are more aerodynamically decoupled from the atmosphere, and retain tight control over

358   their microclimate (Barrera *et al.*, 2000; Korner, 2003), which may reduce inter-tree variability in

359   transpiration.

360   Mean $\delta^{13}C_{cell}$ for each site ranged between -27.2‰ and -26.7‰, (grand mean = -26.6±0.7‰),

361   which is consistent with typical values for C3 land-plants (O'Leary, 1988). Mean inter-tree

362   variability was low (σ range = 0.6 - 0.8‰) and did not vary with altitude or morphology. In this

15

363   case, $\delta^{13}C_{cell}$ variability may be dominated by carbon assimilation rather than stomatal

364   conductance (in support of findings of Farquhar, Barbour and Henry, (1998) and Scheidegger *et*

365   *al.*, (2000) for example). A dominant stomatal conductance signal would lead to co-varying

366   carbon and oxygen isotope ratios with morphology (Lavergne, Daux, *et al.*, 2017; Guerrieri *et*

367   *al.*, 2019).

368   The low EPS in this study means our chronology is inappropriate for studying climate variations

369   at interannual resolution, and would need to be updated with more trees if that were the purpose

370   of the study. However, this does not prevent us using the data to understand a longer-term

371   integrated climate signal; the low inter-tree variability in the prostrate plants in particular

372   suggests they may function well as a record of climate information integrated over longer

373   timescales and we test this hypothesis using a physiological model below. This is particularly

374   pertinent to the fossil plants in this study, where the tree ring widths are extremely narrow and

375   do not provide sufficient material for analysis at annual resolution; data from the fossil plants is

376   integrated over the entire individual plant.

377   *3.2 Carbon isotopes in fossil Nothofagus*

378   Mean $\delta^{13}C_{cell}$ was -22.6±1.9‰ (1 σ). The inter-tree variability here is much larger than in either

379   the arboreal or prostrate plants from Isla Navarino (-26.6±0.7‰), which again is consistent with

380   the dataset spanning millennial timescales. This range of values is significantly enriched by

381   ~+4‰ (p<0.001) relative to the mean values seen in the modern *Nothofagus* trees (Fig. 2).

382   Scarring on the bark (Francis and Hill, 1996) of the fossil plants implies strong winds and

383   paleosol analysis suggests that MAP was 120-220 mm (Retallack, Krull and Bockheim, 2001),

384   which is considerably lower than the MAP on Isla Navarino (400-500 mm). This would lead to

385   enhanced water stress, although fossil *Nothofagus* leaves associated with the wood fragments

386   are large in size indicating that the plants were not living in a marginal habitat (Hill et al., 1996)

387 and thus any water stress could not have been too severe. For both fossil tree age scenarios,

388 atmospheric CO_2 was equal to, or greater than present day, at ~400 ppm for the Pliocene, and

389 exceeding 500 ppm for the warmest periods of the mid-Miocene (Pagani *et al.*, 2010; Holbourn

390 *et al.*, 2015; Levy *et al.*, 2016). Both of these factors would lead to enhanced water-use

391 efficiency, reducing stomatal conductance and hence enriching $\delta^{13}C_{cell}$.

392 Additionally, Tipple, Meyers and Pagani, (2010) (Tipple, Meyers and Pagani, 2010) find that

393 $\delta^{13}C$ of atmospheric CO_2 was higher than present day for both the Pliocene (between around -

394 6.7 and -6‰) and mid-Miocene (between around -5.7 and -5‰), compared to between -8.5 and

395 -7.5‰ for the present day (Keeling *et al.*, 2017). This increase in the baseline $\delta^{13}C$ in

396 combination with enhanced water-use efficiency, is sufficient to explain the large enrichment we

397 see between modern and fossil $\delta^{13}C_{cell}$ data.

398 *3.3. Source water $\delta^{18}O$ in modern Nothofagus*

399 There was no significant trend in $\delta^{18}O$ of measured soil, root or stream waters with altitude,

400 which is most likely because of the small altitude range covered in this study (0-600 m). Sites 2

401 and 3 $\delta^{18}O_{soil}$ are statistically different from each other (p<0.05; one way ANOVA with post-hoc

402 Tukey test) and site 2 is also significantly depleted relative to the stream and root water,

403 suggesting an increased contribution from winter precipitation to soils. From these observations

404 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

405 controlled by latitude, condensation temperature and precipitation amount (Dansgaard, 1964).

406 *3.4 Source water $\delta^{18}O$ prediction from modern cellulose $\delta^{18}O$*

407 We now test whether $\delta^{18}O_{precip}$ can be reconstructed from $\delta^{18}O_{cell}$, using the model from

408 Anderson et al., (2002).

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

410 1.5‰; Fig. 3). This could be due to model parametrization; we chose a value of exactly 27‰ for

411 the biological fraction factor ε, but another value within the accepted range of 24-30‰ could

412 equally be chosen (as in Anderson et al., 2002 and demonstrated by the *x*-axis error bars).

413 Moreover, modelled $\delta^{18}O_{source}$ was not statistically different from $\delta^{18}O_{root}$, $\delta^{18}O_{stream}$ or GNIP

414 summer precipitation, indicating that the model works well for predicting $\delta^{18}O_{precip}$ from

415 measured $\delta^{18}O_{cell}$ in *Nothofagus*. We now apply the model to the fossil *Nothofagus* in order to

416 calculate ancient $\delta^{18}O_{precip}$.

417 *3.5. Reconstructing ancient precipitation $\delta^{18}O$ from fossil Nothofagus*

418 Mean $\delta^{18}O_{cell}$ for the fossil plants was 20.3±3.0‰. The inter-tree variability is similar in

419 magnitude to that seen in modern trees, but is greater than the inter-tree variability seen in the

420 prostrate plants of this study. It seems likely that these data capture both significant temporal

421 variability and climate variability. It is important to note that here, we are treating all fossils as

422 being geologically contemporaneous as they were all collected from the same bed, but it is

423 highly likely that our data may span multiple millennia. Ice sheet fluctuations during both the

424 mid-Miocene and Pliocene occurred at orbital timescales (Greenop *et al.*, 2014; Patterson *et al.*,

425 2014); therefore the duration represented by the fossils must be less than 100 kyr, but long

426 enough for poorly developed soils to form and woody plants to colonise the area. This is

427 consistent with the larger variability in the fossil data compared to the modern. Mean $\delta^{18}O_{cell}$ for

428 the Sirius Group plant is significantly depleted by ~5‰ (p<0.001) relative to the mean of the

429 modern *Nothofagus* trees from Isla Navarino (25.5±1.5‰). Broadly, there are two major controls

430 on $\delta^{18}O_{cell}$, which could cause such an offset: evapotranspiration rates (controlled by relative

431 humidity and stomatal conductance) and $\delta^{18}O$ of the plant's source water (McCarroll and

432 Loader, 2004). From the modern data, we assume that plant source water is equal to

433 precipitation $\delta^{18}O$ within the uncertainty of precipitation variability. In addition to latitude,

18

434   precipitation amount, and temperature, there are further processes that could modify this signal,

435   including evaporation from soil or plants using groundwater as a moisture source. Depletion

436   could result from a large decrease in stomatal conductance caused by increased vapour

437   pressure deficit reducing evapotranspiration from leaves. However, vapour pressure deficit

438   across southern Chile is already relatively low (<0.5 kPa; Du *et al.*, 2018) and it is unlikely that

439   there would have been significant decreases in vapour pressure deficit for Antarctica when

440   summer temperatures are not predicted to have been much lower (Rees-Owen *et al.*, 2018).

441   Alternatively, decreased $\delta^{18}O_{cell}$ could be caused by a difference in $\delta^{18}O_{precip}$, which is consistent

442   with the higher palaeolatitude of the fossil plants (85°S for the Sirius Group, 54°S for Isla

443   Navarino). We test this hypothesis using the physiological model for $\delta^{18}O_{precip}$ from Anderson et

444   al, (2002). Using this approach, we calculate that mean continental Antarctic palaeoprecipitation

445   was -16±4.2‰ (1 σ; ranging between -26‰ and -3.5‰). Since $\delta^{18}O_{cell}$ is strongly modified by

446   ambient relative humidity, the large range in our results is consistent with the conservative (i.e.

447   wide) humidity range used in this study.

448   In the present day, $\delta^{18}O_{precip}$ over East Antarctica is highly variable, ranging from -55‰ at the

449   highest elevations and furthest from the coast, to -25‰ near sea level at lower latitudes <75°S

450   (Masson-Delmotte *et al.*, 2008). However, there is considerable uncertainty surrounding the

451   palaeoaltitude of the *Nothofagus* fossils sampled in this study (Ackert, Jr. and Kurz, 2004),

452   which makes it difficult to provide context for the reconstructed $\delta^{18}O_{precip}$ values. We therefore

453   compared our record to measured

454   Antarctic $\delta^{18}O_{precip}$ from sites above 75°S and less than 700 m above sea level (the height of the

455   timberline on Isla Navarino; Masson-Delmotte et al., 2008), representing a reasonable habitat

456   range. Reconstructed $\delta^{18}O_{precip}$ was significantly enriched by ~+12‰ relative to modern $\delta^{18}O_{precip}$

457   (ancient mean = -16‰, modern mean = -28‰; p <0.001; Fig. 4). Growth experiments have

19

458   suggested that plant δ^2H (and therefore by extension, δ^{18}O) can be significantly enriched in

459   plants grown under continuous light, analogous to the polar light regime (Yang *et al.*, 2009).

460   Therefore, part of the enrichment in the Sirius Group specimens could be accounted for by the

461   continuous light regime experienced by the Antarctic plants during the growing season, which

462   would increase $\delta^{18}O_{cell}$ via continuous transpiration, as opposed to the light regime on Isla

463   Navarino, where plants undergo a diurnal transpiration-respiration cycle. However, the plants

464   used by Yang et al. (2009) have a relatively high transpiration rate because of the relatively

465   warm growing temperatures used in their experimental study. We suggest that the transpiration

466   rate for the Sirius Group plants would likely be much lower because of the cold summer

467   temperatures ($\sim$5°C, compared to $\sim$20°C in the environment used by Yang et al., 2009).

468   Furthermore, *Nothofagus* have been documented as having significantly tighter stomatal control

469   of transpiration than co-existing conifers (Fernández, Gyenge and Schlichter, 2009), as used by

470   Yang et al. (2009). Therefore it seems likely that there is much lower enrichment due to

471   continuous light in the Sirius Group fossils (see Supplementary Information for further

472   discussion).

473   Our result has implications for regional and global climate during periods of ice sheet retreat in

474   the Neogene. A significant enrichment in precipitation isotopes implies a considerable change in

475   some of the atmospheric processes of the hydrological cycle. Plausible mechanisms include

476   increased temperatures affecting fractionation during condensation, or changes in rainout

477   patterns due to shifts in source moisture region or different atmospheric circulation patterns

478   leading to a shortened vapour transport pathway. As previously discussed, warmer Antarctic

479   temperatures (relative to today) are consistent with multiple contemporaneous terrestrial

480   temperature proxies, which suggest that summer temperatures reached 5°C during the period of

481   study (Ashworth and Kuschel, 2003; Ashworth and Preece, 2003; Ashworth and Cantrill, 2004;

482   Rees-Owen *et al.*, 2018). This result is also consistent with both age scenarios for the site:

483   during both the mid-Miocene and Pliocene, sea surface temperatures in the Southern Ocean

484   were several degrees warmer than today (Warny *et al.*, 2009; McKay *et al.*, 2012; Clark *et al.*,

485   2013) and there is evidence for reduced sea ice cover (Whitehead, Wotherspoon and Bohaty,

486   2005; Warny *et al.*, 2009). However, previous work by Feakins *et al,* (2012) suggests that the

487   relationship between temperature and precipitation isotopes earlier in the Miocene (20 - 15 Ma)

488   on the Antarctic coast was different from the modern, driven by increased evaporation from a

489   warmer Southern Ocean. Similarly, isotopic disequilibrium between vapour and precipitation in

490   modern-day Patagonia has been suggested to explain greater than expected $\delta^{18}O_{cell}$

491   (Penchenat *et al.*, 2020). This implies that other factors may also influence the hydrological

492   cycle at this time, which is plausible within the context of a warmer Neogene world, where

493   warmer Southern Ocean temperatures could drive an increase in evaporation from high latitude

494   moisture sources. Equally, the smaller ice sheet could well have influenced regional

495   atmospheric circulation patterns, and changes in global atmospheric circulation are documented

496   for the Pliocene (Brierley et al., 2009). These variables are likely to be important for

497   understanding the full significance of our data, but are unconstrained, and a full exploration of

498   hydrological changes is beyond the scope of this study. These questions could be more fully

499   answered through further data collection to reduce proxy uncertainty, and the use of a coupled

500   ocean-atmosphere climate model to investigate hydrodynamic changes.

501   **4. Conclusions**

502   By testing a simple physiological model linking $\delta^{18}O_{cell}$ with $\delta^{18}O_{precip}$ in two species of modern

503   *Nothofagus* plants, which grow in both arboreal and prostrate form, we found that $\delta^{18}O_{cell}$ of

504   prostrate *Nothofagus* faithfully records $\delta^{18}O_{precip}$ at multi- year resolution. Hitherto, most tree ring

505   stable isotope analyses have been applied to trees with an arboreal habit in temperate and

506   tropical environments. Therefore, it was previously unclear whether the assumptions made in

507   tree ring isotope theory hold true for *krummholz*-type plants, such as those from Oliver Bluffs,

508   which feature growth asymmetry that could affect isotopic signals via resource partitioning. Our

509   findings demonstrate that prostrate trees are potentially suitable archives for recording

510   climatological means over longer periods (on the order of decades). This result opens up high

511   latitude and altitude end-member environments in both palaeo and modern times for tree ring

512   isotope analysis.

513   The carbon isotope composition of cellulose from exceptionally well-preserved Neogene fossil

514   wood from the Transantarctic Mountains, Antarctica was ~4‰ more positive than that of the

515   modern samples. This difference is best explained as the result of a more positive value for the

516     $\delta^{13}C$ of contemporaneous atmospheric CO_2 and enhanced water use efficiency at the Oliver

517   Bluffs site, although the precise contribution of each of these factors to this signal is unknown.

518   The oxygen isotopic composition of the fossil wood provides new insights into Neogene

519   hydrological cycling. Our record indicates that during a period of EAIS ice sheet retreat in which

520   small prostrate shrubs colonised the exposed glacial landscape close to the South Pole, the

521   hydrological cycle was markedly different to today with precipitation significantly enriched in ^{18}O

522   by ~12‰ relative to modern precipitation over the continent. While the enrichment may be

523   temperature driven alone, our result correlates well with the result of Feakins et al. (2012),

524   suggesting that moisture source regions may have been different in the past. However, it is not

525   possible to distinguish between these two possibilities, or some combination of both, based on

526   the geochemical data alone.

542

543

544

545

546

547

548

References

549   **References**

550   Ackert, Jr., R. P. and Kurz, M. D. (2004) 'Age and uplift rates of Sirius Group sediments in the

551   Dominion Range , Antarctica , from surface exposure dating and geomorphology', *Global and*

552   *Planetary Change*, 42, pp. 207–225. doi: 10.1016/j.gloplacha.2004.02.001.

553   Aggarwal, P. K. *et al.* (2012) 'Stable isotopes in global precipitation: A unified interpretation

554   based on atmospheric moisture residence time', *Geophysical Research Letters*, 39(11), p. n/a-

555   n/a. doi: 10.1029/2012GL051937.

556   Allison, G. B., Gat, J. R. and Leaney, F. W. J. (1985) 'The relationship between deuterium and

557   oxygen-18 delta values in leaf water', *Chemical Geology (Isotope Geoscience Section)*, 58, pp.

558   145–156.

559   Anderson, W. T. *et al.* (2002) 'Model evaluation for reconstructing the oxygen isotopic

560   composition in precipitation from tree ring cellulose over the last century', *Chemical Geology*,

561   182(2–4), pp. 121–137. doi: 10.1016/S0009-2541(01)00285-6.

562   Arens, N. C., Jahren, A. H. and Amundson, R. (2000) 'Can C3 plants faithfully record the carbon

563   isotopic composition of atmospheric carbon dioxide?', *Paleobiology*, 26(1), pp. 137–164. doi:

564   10.1666/0094-8373(2000)026<0137:ccpfrt>2.0.co;2.

565   Ashworth, A. C. and Cantrill, D. J. (2004) 'Neogene vegetation of the Meyer Desert Formation

566   (Sirius Group) Transantarctic Mountains, Antarctica', *Palaeogeography, Palaeoclimatology,*

567   *Palaeoecology*, 213(1–2), pp. 65–82. doi: 10.1016/j.palaeo.2004.07.002.

568   Ashworth, A. C. and Kuschel, G. (2003) 'Fossil weevils (Coleoptera: Curculionidae) from latitude

569   85°S Antarctica', *Palaeogeography, Palaeoclimatology, Palaeoecology*, 191(2), pp. 191–202.

570   doi: 10.1016/S0031-0182(02)00712-5.

571   Ashworth, A. C. and Preece, R. C. (2003) 'The first freshwater molluscs from Antarctica',

572   *Journal Molluscan Studies*, 69(1), pp. 89–92. doi: 10.1093/mollus/69.1.89.

573   Austermann, J. *et al.* (2015) 'The impact of dynamic topography change on Antarctic ice sheet

574   stability during the mid-Pliocene warm period', *Geology*, 43(10), pp. 927–930. doi:

575   10.1130/G36988.1.

576   Baldini, L. M. *et al.* (2008) 'Spatial variability in the European winter precipitation δ 18 O-NAO

577   relationship: Implications for reconstructing NAO-mode climate variability in the Holocene',

578   *Geophysical Research Letters*, 35(4), p. L04709. doi: 10.1029/2007GL032027.

579   Ballantyne, A. P. *et al.* (2006) 'Pliocene Arctic temperature constraints from the growth rings and

580   isotopic composition of fossil larch', *Palaeogeography Palaeoclimatology Palaeoecology*,

581   242(3–4), pp. 188–200.

582   Ballantyne, A. P. *et al.* (2010) 'Significantly warmer Arctic surface temperatures during the

583   Pliocene indicated by multiple independent proxies', *Geology*, 38(7), pp. 603–606. doi:

584   10.1130/G30815.1.

585   Barrera, M. D. *et al.* (2000) 'Structural and functional changes in Nothofagus pumilio forests

586   along an altitudinal gradient in Tierra del Fuego, Argentina', *Journal of Vegetation Science*, 11,

587   pp. 179--188. doi: 10.2307/3236797.

588   Barrett, P. J. (2013) 'Resolving views on Antarctic Neogene glacial history – the Sirius debate',

589   *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 104(May

590   2013), pp. 31–53. doi: 10.1017/S175569101300008X.

591   Battipaglia, G. *et al.* (2013) 'Elevated CO2 increases tree-level intrinsic water use efficiency:

592   insights from carbon and oxygen isotope analyses in tree rings across three forest FACE sites.',

593   *New Phytologist*, 197(2), pp. 544–54. doi: 10.1111/nph.12044.

594   Beerling, D. J. (1996) 'Ecophysiological responses of woody plants to past CO2 concentrations',

595   *Tree Physiology*, 16(4), pp. 389–396. doi: 10.1093/treephys/16.4.389.

596   Brienen, R. J. W. *et al.* (2012) 'Oxygen isotopes in tree rings are a good proxy for Amazon

597   precipitation and El Nino-Southern Oscillation variability', *Proceedings of the National Academy*

598   *of Sciences*, 109(42), pp. 16957–16962. doi: 10.1073/pnas.1205977109.

599   Brierley, C. M. *et al.* (2009) 'Greatly Expanded Tropical Warm Pool and Weakened Hadley

600   Circulation in the Early Pliocene', *Science*, 323(5922).

601   Buhay, W. M., Edwards, T. W. D. and Aravena, R. (1996) 'Evaluating kinetic fractionation

602   factors used for ecologic and paleoclimatic reconstructions from oxygen and hydrogen isotope

603   ratios in plant water and cellulose', *Geochimica et Cosmochimica Acta*, 60(12), pp. 2209–2218.

604   doi: 10.1016/0016-7037(96)00073-7.

605   Buras, A. and Wilmking, M. (2014) 'Straight lines or eccentric eggs? A comparison of radial and

606   spatial ring width measurements and its implications for climate transfer functions',

607   *Dendrochronologia*. Elsevier GmbH., 32(4), pp. 313–326. doi: 10.1016/j.dendro.2014.07.002.

608   Burckle, L. H. and Potter, N. (1996) 'Pliocene-Pleistocene diatoms in Paleozoic and Mesozoic

609   sedimentary and igneous rocks from Antarctica : A Sirius problem solved Pliocene-Pleistocene

610   diatoms in Paleozoic and Mesozoic sedimentary and igneous rocks from Antarctica : A Sirius

611   problem solved', *Geology*, 24, pp. 235–238. doi: 10.1130/0091-7613(1996)024<0235.

612   Carlquist, S. (1987) 'Pliocene Nothofagus wood from the Transantarctic mountains', *Aliso*, 11,

613   pp. 571–583.

614   Cernusak, L. A. *et al.* (2007) 'Transpiration efficiency of a tropical pioneer tree (Ficus insipida) in

615   relation to soil fertility', *Journal of Experimental Botany*, 58(13), pp. 3549–3566. doi:

616   10.1093/jxb/erm201.

617   Cernusak, L. A. and English, N. B. (2014) 'Beyond tree-ring widths: Stable isotopes sharpen the

618   focus on climate responses of temperate forest trees', *Tree Physiology*, 35(1), pp. 1–3. doi:

619   10.1093/treephys/tpu115.

620   Cernusak, L. A., Winter, K. and Turner, B. L. (2009) 'Physiological and isotopic (δ13C and

621   δ18O) responses of three tropical tree species to water and nutrient availability', *Plant, Cell &*

622   *Environment*, 32(10), pp. 1441–1455. doi: 10.1111/j.1365-3040.2009.02010.x.

623   Clark, N. A. *et al.* (2013) 'Fossil proxies of near-shore sea surface temperatures and seasonality

624   from the late Neogene Antarctic shelf', *Naturwissenschaften*, 100, pp. 699–722. doi:

625   10.1007/s00114-013-1075-9.

626   Cook, C. P. *et al.* (2013) 'Dynamic behaviour of the East Antarctic ice sheet during Pliocene

627   warmth', *Nature Geoscience*, 6(9), pp. 765–769. doi: 10.1038/ngeo1889.

628   Csank, A. Z. *et al.* (2011) 'Climate variability in the Early Pliocene Arctic: Annually resolved

629   evidence from stable isotope values of sub-fossil wood, Ellesmere Island, Canada',

630   *Palaeogeography Palaeoclimatology Palaeoecology*, 308(3–4), pp. 339–349.

631   Cullen, L. E. and Grierson, P. F. (2009) 'Multi-decadal scale variability in autumn-winter rainfall

632   in south-western Australia since 1655 AD as reconstructed from tree rings of Callitris

633   columellaris', *Climate Dynamics*, 33(2–3), pp. 433–444. doi: 10.1007/s00382-008-0457-8.

634   Danis, P.-A. *et al.* (2012) 'MAIDENiso: a multiproxy biophysical model of tree-ring width and

635   oxygen and carbon isotopes', *Canadian Journal of Forest Research*, 42(9), pp. 1697–1713. doi:

636   10.1139/x2012-089.

637 Dansgaard, W. (1964) 'Stable isotopes in precipitation', *Tellus*, 16(4), pp. 436–468. doi:

638 10.3402/tellusa.v16i4.8993.

639 Daux, V. *et al.* (2018) 'Comparisons of the Performance of δ 13 C and δ 18 O of Fagus sylvatica

640 , Pinus sylvestris , and Quercus petraea in the Record of Past Climate Variations', *Journal of*

641 *Geophysical Research: Biogeosciences*, 123(4), pp. 1145–1160. doi: 10.1002/2017JG004203.

642 Dolan, A. M. *et al.* (2011) 'Sensitivity of Pliocene ice sheets to orbital forcing', *Palaeogeography,*

643 *Palaeoclimatology, Palaeoecology*, 309(1), pp. 98–110. doi: 10.1016/j.palaeo.2011.03.030.

644 Du, J. *et al.* (2018) 'Global Satellite Retrievals of the Near-Surface Atmospheric Vapor Pressure

645 Deficit from AMSR-E and AMSR2', *Remote Sensing*, 10(8), p. 1175. doi: 10.3390/rs10081175.

646 Ehleringer, J. R. *et al.* (1986) 'Leaf carbon isotope and mineral composition in subtropical plants

647 along an irradiance cline', *Oecologia*, 70(4), pp. 520–526. doi: 10.1007/BF00379898.

648 Farquhar, G. D., Barbour, M. M. and Henry, B. K. (1998) 'Interpretation of oxygen isotope

649 composition of leaf material.', *Stable Isotopes: Integration of Biological, Ecological, and*

650 *Geochemical Processes*, pp. 27–48.

651 Farquhar, G. D., Ehleringer, J. R. and Hubick, K. T. (1989) 'Carbon isotope discrimination and

652 photosynthesis', *Annual Review of Plant Physiology and Plant Molecular Biology*, 40, pp. 503–

653 537.

654 Farquhar, G. D. and Gan, K. S. (2003) 'On the progressive enrichment of the oxygen isotopic

655 composition along a leaf', *Plant Cell Environment*, 26(6), pp. 801–819.

656 Feakins, Sarah J., Warny, S. and Lee, J.-E. (2012) 'Hydrologic cycling over Antarctica during

657 the middle Miocene warming - Supplementary Information', *Nature Geoscience*, 5(8), pp. 557–

658 560. doi: 10.1038/ngeo1498.

659 Feakins, Sarah J, Warny, S. and Lee, J. (2012) 'Hydrologic cycling over Antarctica during the

660 middle Miocene warming', *Nature Geoscience*. Nature Publishing Group, 5(8), pp. 557–560. doi:

661 10.1038/ngeo1498.

662 Fernández, M. E., Gyenge, J. and Schlichter, T. (2009) 'Water flux and canopy conductance of

663 natural versus planted forests in Patagonia, South America', *Trees - Structure and Function*,

664 23(2), pp. 415–427. doi: 10.1007/s00468-008-0291-y.

665 Fielding, C. R. *et al.* (2012) 'Neogene stratigraphy of Taylor Valley, Transantarctic Mountains,

666 Antarctica: Evidence for climate dynamism and a vegetated Early Pliocene coastline of

667 McMurdo Sound', *Global and Planetary Change*, 96–97, pp. 97–104. doi:

668 10.1016/j.gloplacha.2010.09.003.

669 Francis, J. E. and Hill, R. S. (1996) 'Fossil Plants from the Pliocene Sirius Group ,

670 Transantarctic Evidence for Mountains : Climate from Growth Rings and Fossil Leaves',

671 *PALAIOS*, 11(4), pp. 389–396.

672 Gagen, M. *et al.* (2007) 'Exorcising the `segment length curse': summer temperature

673 reconstruction since AD 1640 using non-detrended stable carbon isotope ratios from pine trees

674 in northern Finland', *The Holocene*, 17(4), pp. 435–446. doi: 10.1177/0959683607077012.

675 Gasson, E. *et al.* (2016) 'Dynamic Antarctic ice sheet during the early to mid-Miocene',

676 *Proceedings of the National Academy of Sciences*, (34), p. 201516130. doi:

677 10.1073/pnas.1516130113.

678 Gessler, A. *et al.* (2007) 'Oxygen isotope enrichment of organic matter in Ricinus communis

679 during the diel course and as affected by assimilate transport', *New Phytologist*, 174(3), pp.

680 600–613. doi: 10.1111/j.1469-8137.2007.02007.x.

681   Gessler, A. *et al.* (2009) 'Tracing carbon and oxygen isotope signals from newly assimilated

682   sugars in the leaves to the tree-ring archive.', *Plant, cell & environment*, 32(7), pp. 780–95. doi:

683   10.1111/j.1365-3040.2009.01957.x.

684   Gessler, A. *et al.* (2014) 'Stable isotopes in tree rings : towards a mechanistic understanding of

685   isotope fractionation and mixing processes from the leaves to the wood', *Tree Physiology*, 00,

686   pp. 1–23. doi: 10.1093/treephys/tpu040.

687   Greenop, R. *et al.* (2014) 'Middle Miocene climate instability associated with high-amplitude CO

688   2 variability', *Paleoceanography*, 29(9), pp. 845–853. doi: 10.1002/2014PA002653.

689   Griener, K. W. *et al.* (2015) 'Early to middle Miocene vegetation history of Antarctica supports

690   eccentricity-paced warming intervals during the Antarctic icehouse phase', *Global and Planetary*

691   *Change*. Elsevier B.V., 127, pp. 67–78. doi: 10.1016/j.gloplacha.2015.01.006.

692   Grießinger, J. *et al.* (2018) 'Imprints of climate signals in a 204 year δ18O tree-ring record of

693   nothofagus pumilio from Perito Moreno Glacier, Southern Patagonia (50◦S)', *Frontiers in Earth*

694   *Science*, 6(April), pp. 1–17. doi: 10.3389/feart.2018.00027.

695   Grissino-Mayer, H. D. (2001) 'Evaluating crossdating accuracy: a manual and tutorial for the

696   computer program COFECHA'.

697   Guerrieri, R. *et al.* (2019) 'Disentangling the role of photosynthesis and stomatal conductance

698   on rising forest water-use efficiency', *Proceedings of the National Academy of Sciences*,

699   116(34), pp. 16909–16914. doi: 10.1073/pnas.1905912116.

700   Hantemirov, R., Shiyatov, S. and Gorlanova, L. (2011) 'Dendroclimatic study of Siberian

701   juniper', *Dendrochronologia*, 29(2), pp. 119–122. doi: 10.1016/j.dendro.2010.05.001.

702   Hare, V. J. *et al.* (2018) 'Atmospheric CO2 effect on stable carbon isotope composition of

703   terrestrial fossil archives', *Nature Communications*, 9(1), p. 252. doi: 10.1038/s41467-017-

704   02691-x.

705   Harwood, D. M. (1986) *Diatom biostratigraphy and paleoecology with a Cenozoic history of*

706   *Antarctic ice sheets*. Ohio State University.

707   Hill, R. S., Harwood, D. M. and Webb, P.-N. (1996) 'Nothofagus beardmorensis

708   (Nothofagaceae), a new species based on leaves from the Pliocene Sirius Group,

709   Transantarctic Mountains, Antarctica', *Review of Palaeobotany and Palynology*, 94, pp. 11–24.

710   Hill, R. S. and Jordan, G. J. (1996) 'Macrofossils as indicators of Plio-Pleistocene climates in

711   Tasmaniaand Antarctica', *Papers and Proceedings of the Royal Society of Tasmania*, 130(2),

712   pp. 9–15.

713   Hill, S. A. *et al.* (1995) 'Rapid recycling of triose phosphates in oak stem tissue', *Plant, Cell &*

714   *Environment*, 18(8), pp. 931–936.

715   Holbourn, A. *et al.* (2015) 'Global perturbation of the carbon cycle at the onset of the Miocene

716   Climatic Optimum', *Geology*, 43(2), pp. 123–126. doi: 10.1130/G36317.1.

717   Holmes, R. L. (1983) 'Computer-assisted quality control in tree-ring dating and measurement',

718   *Tree-Ring Bulletin*, 43(1), pp. 69–78.

719   Hook, B. A. *et al.* (2014) 'Stable isotope paleoclimatology of the earliest Eocene using

720   kimberlite-hosted mummified wood from the Canadian Subarctic', *Biogeosciences Discussions*,

721   11(11), pp. 16269–16308. doi: 10.5194/bgd-11-16269-2014.

722   Hook, B. A. *et al.* (2015) 'Extraction of α-cellulose from mummified wood for stable isotopic

723   analysis', *Chemical Geology*, 405, pp. 19–27. doi: 10.1016/j.chemgeo.2015.04.003.

724   Hunsinger, G. B., Hagopian, W. M. and Jahren, A. H. (2010) 'Offline oxygen isotope analysis of

725   organic compounds with high N:O', *Rapid Communications in Mass Spectrometry*, 24(21), pp.

726   3182–3186. doi: 10.1002/rcm.4752.

727   Jahren, A. H. *et al.* (2001) 'Terrestrial record of methane hydrate dissociation in the Early

728   Cretaceous', *Geology*, 29(2), pp. 159–162. doi: 10.1130/0091-

729   7613(2001)029<0159:TROMHD>2.0.CO.

730   Jahren, A. H. *et al.* (2009) 'The environmental water of the middle Eocene Arctic: Evidence from

731   δD, δ18O and δ13C within specific compounds', *Palaeogeography Palaeoclimatology*

732   *Palaeoecology*. Elsevier B.V., 271(1–2), pp. 96–103. doi: 10.1016/j.palaeo.2008.09.016.

733   Jahren, A. H. and Sternberg, L. S. L. (2008) 'Annual patterns within tree rings of the Arctic

734   middle Eocene (ca. 45 Ma): Isotopic signatures of precipitation, relative humidity, and

735   deciduousness', *Geology*, 36(2), pp. 99–102. doi: Doi 10.1130/G23876a.1.

736   Keeling, R. F. *et al.* (2017) 'Atmospheric evidence for a global secular increase in carbon

737   isotopic discrimination of land photosynthesis', *Proceedings of the National Academy of*

738   *Sciences*, 114(39), pp. 10361–10366. doi: 10.1073/pnas.1619240114.

739   Köhler, I. H. *et al.* (2010) 'Intrinsic water-use efficiency of temperate seminatural grassland has

740   increased since 1857: an analysis of carbon isotope discrimination of herbage from the Park

741   Grass Experiment', *Global Change Biology*, 16(5), pp. 1531–1541. doi: 10.1111/j.1365-

742   2486.2009.02067.x.

743   Korner, C. (2003) 'Plant ecology at high elevations', in *Alpine plant life: functional plant ecology*

744   *of high mountain ecosystems*, pp. 1–7.

745   Kress, A. *et al.* (2010) 'A 350 year drought reconstruction from Alpine tree ring stable isotopes',

746   *Global Biogeochemical Cycles*, 24(2), p. n/a-n/a. doi: 10.1029/2009GB003613.

747   Labuhn, I. *et al.* (2016) 'French summer droughts since 1326 CE: a reconstruction based on

748   tree ring cellulose δ 18 O', *Climate of the Past*, 12(5), pp. 1101–1117. doi: 10.5194/cp-12-1101-

749   2016.

750   Lavergne, A. *et al.* (2016) 'Are the oxygen isotopic compositions of *Fitzroya cupressoides* and

751   *Nothofagus pumilio* cellulose promising proxies for climate reconstructions in northern

752   Patagonia?', *Journal of Geophysical Research: Biogeosciences*, p. n/a-n/a. doi:

753   10.1002/2015JG003260.

754   Lavergne, A., Daux, V., *et al.* (2017) 'Improvement of isotope-based climate reconstructions in

755   Patagonia through a better understanding of climate influences on isotopic fractionation in tree

756   rings', *Earth and Planetary Science Letters*, 459, pp. 372–380. doi: 10.1016/j.epsl.2016.11.045.

757   Lavergne, A., Gennaretti, F., *et al.* (2017) 'Modelling tree ring cellulose d18O variations in two

758   temperature-sensitive tree species from North and South America', *Climate of the Past*, 13(11),

759   pp. 1515–1526. doi: 10.5194/cp-13-1515-2017.

760   Lavergne, A. *et al.* (2018) 'Past Summer Temperatures Inferred From Dendrochronological

761   Records of <scp> *Fitzroya cupressoides* </scp> on the Eastern Slope of the Northern

762   Patagonian Andes', *Journal of Geophysical Research: Biogeosciences*, 123(1), pp. 32–45. doi:

763   10.1002/2017JG003989.

764   Lawver, L. A. and Gahagan, L. M. (2003) 'Evolution of Cenozoic seaways in the circum-

765   Antarctic region', *Palaeogeography, Palaeoclimatology, Palaeoecology*, 198(1–2), pp. 11–37.

766   doi: 10.1016/S0031-0182(03)00392-4.

767   Levy, R. H. *et al.* (2016) 'Antarctic ice sheet sensitivity to atmospheric CO2 variations in the

early to mid-Miocene', *Proceedings of the National Academy of Sciences*, 113(13), pp. 3453–
3458. doi: 10.1073/pnas.1516030113.

Majoube, M. (1971) 'Oxygen-18 and deuterium fractionation between water and steam', *Journal de Chimie Physique et de Physico-Chimie Biologique*, 68(10), p. 1423.

Masson-Delmotte, V. *et al.* (2008) 'A Review of Antarctic Surface Snow Isotopic Composition: Observations, Atmospheric Circulation, and Isotopic Modeling*', *Journal of Climate*, 21(13), pp. 3359–3387. doi: 10.1175/2007JCLI2139.1.

McCarroll, D. and Loader, N. J. (2004) 'Stable isotopes in tree rings', *Quaternary Science Reviews*, 23, pp. 771–801.

McKay, R. M. *et al.* (2012) 'Antarctic and Southern Ocean influences on Late Pliocene global cooling.', *Proceedings of the National Academy of Sciences of the United States of America*, 109(17), pp. 6423–8. doi: 10.1073/pnas.1112248109.

McKelvey, B. C. *et al.* (1991) 'The Dominion Range Sirius Group: a record of the late Pliocene-early Pleistocene Beardmore Glacier', in *Geological Evolution of Antarctica*, pp. 675–682.

Mercer, J. H. (1986) 'Southernmost Chile : A modern analog of the southern shores of the Ross Embayment during Pliocene warm intervals', *Antarctic Journal of the United States*, 21(5), pp. 103–105.

Moore, D. M. (1983) *Flora of Tierra del Fuego*. Anthony Nelson, Oswesrty.

Nabeshima, E. *et al.* (2018) 'Seasonal changes of δD and δ18O in tree-ring cellulose of Quercus crispula suggest a change in post-photosynthetic processes during earlywood growth', *Tree Physiology*. Edited by M. Mencuccini, 38(12), pp. 1829–1840. doi: 10.1093/treephys/tpy068.

790 Naulier, M. *et al.* (2014) 'Carbon and oxygen isotopes of lakeshore black spruce trees in

791 northeastern Canada as proxies for climatic reconstruction', *Chemical Geology*, 374–375, pp.

792 37–43. doi: 10.1016/j.chemgeo.2014.02.031.

793 O'Leary, M. H. (1988) 'Carbon isotopes in photosynthesis', *BioScience*, 38(5), pp. 328–336.

794 Offerman, C. *et al.* (2011) 'The long way down - are carbon and oxygen isotope signals in the

795 tree ring uncoupled from canpoy physiological processes?', *Tree Physiology*, 31(10), pp. 1088–

796 1102.

797 Ogée, J. *et al.* (2003) 'MuSICA, a CO2, water and energy multilayer, multileaf pine forest model:

798 evaluation from hourly to yearly time scales and sensitivity analysis', *Global Change Biology*,

799 9(5), pp. 697–717. doi: 10.1046/j.1365-2486.2003.00628.x.

800 Ogée, J. *et al.* (2009) 'A single-substrate model to interpret intra-annual stable isotope signals in

801 tree-ring cellulose', *Plant, Cell & Environment*, 32(8), pp. 1071–1090. doi: 10.1111/j.1365-

802 3040.2009.01989.x.

803 Ohneiser, C. *et al.* (2020) 'Warm fjords and vegetated landscapes in early Pliocene East

804 Antarctica', *Earth and Planetary Science Letters*. Elsevier B.V., 534, p. 116045. doi:

805 10.1016/j.epsl.2019.116045.

806 Pagani, M. *et al.* (2010) 'High Earth-system climate sensitivity determined from Pliocene carbon

807 dioxide concentrations', *Nature Geoscience*. Nature Publishing Group, 3(1), pp. 27–30. doi:

808 10.1038/ngeo724.

809 Patterson, M. O. *et al.* (2014) 'Orbital forcing of the East Antarctic ice sheet during the Pliocene

810 and Early Pleistocene', *Nature Geosci*, 7(11), pp. 841–847. doi: 10.1038/ngeo2273.

811 Penchenat, T. *et al.* (2020) 'Isotopic Equilibrium Between Precipitation and Water Vapor in

812 Northern Patagonia and Its Consequences on δ 18 O cellulose Estimate', *Journal of*

813 *Geophysical Research: Biogeosciences*, 125(3). doi: 10.1029/2019JG005418.

814 Pollard, D. and Deconto, R. M. (2016) 'Contribution of Antarctica to past and future sea-level

815 rise', *Nature*. Nature Publishing Group, 531(7596), pp. 591–597. doi: 10.1038/nature17145.

816 Pollard, D., Deconto, R. M. and Alley, R. B. (2015) 'Potential Antarctic Ice Sheet retreat driven

817 by hydrofracturing and ice cliff failure', *Earth and Planetary Science Letters*. Elsevier B.V., 412,

818 pp. 112–121. doi: 10.1016/j.epsl.2014.12.035.

819 Pound, M. J. *et al.* (2012) 'Global vegetation dynamics and latitudinal temperature gradients

820 during the Mid to Late Miocene (15.97–5.33Ma)', *Earth-Science Reviews*, 112(1–2), pp. 1–22.

821 doi: 10.1016/j.earscirev.2012.02.005.

822 Priault, P., Wegener, F. and Werner, C. (2009) 'Pronounced differences in diurnal variation of

823 carbon isotope composition of leaf respired CO 2 among functional groups', *New Phytologist*,

824 181(2), pp. 400–412. doi: 10.1111/j.1469-8137.2008.02665.x.

825 Rees-Owen, R. L. *et al.* (2018) 'The last forests on Antarctica: Reconstructing flora and

826 temperature from the Neogene Sirius Group, Transantarctic Mountains', *Organic Geochemistry*,

827 118, pp. 4–14. doi: 10.1016/j.orggeochem.2018.01.001.

828 Retallack, G. J., Krull, E. S. and Bockheim, J. G. (2001) 'New grounds for reassessing

829 palaeoclimate of the Sirius Group, Antarctica', *Journal of the Geological Society, London*, 158,

830 pp. 925–935.

831 Roden, J. S. and Ehleringer, J. R. (1999) 'Observations of hydrogen and oxygen isotopes in leaf

832 water confirm the Craig-Gordon model under wide-ranging environmental conditions', *Plant*

833 *Physiology*, 120(4), pp. 1165–1174.

834   Roden, J. S. and Ehleringer, J. R. (2000) 'Hydrogen and oxygen isotope ratios of tree ring

835   cellulose for field-grown riparian trees', *Oecologia*, 123(4), pp. 481–489. doi:

836   10.1007/s004420000349.

837   Roden, J. S., Lin, G. and Ehleringer, J. R. (2000) 'A mechanistic model for interpretation of

838   hydrogen and oxygen isotope ratios in tree-ring cellulose', *Geochimica et Cosmochimica Acta*,

839   64(1), pp. 21–35. doi: 10.1016/S0016-7037(99)00195-7.

840   Scheidegger, Y. *et al.* (2000) 'Linking stable oxygen and carbon isotopes with stomatal

841   conductance and photosynthetic capacity: a conceptual model', *Oecologia*, 125(3), pp. 350–

842   357. doi: 10.1007/s004420000466.

843   Schubert, B. A. *et al.* (2012) 'A summertime rainy season in the Arctic forests of the Eocene',

844   *Geology*, 40(6), pp. 523–526. doi: 10.1130/G32856.1.

845   Schubert, B. A. and Jahren, A. H. (2011) 'Quantifying seasonal precipitation using high-

846   resolution carbon isotope analyses in evergreen wood', *Geochimica Et Cosmochimica Acta*,

847   75(22), pp. 7291–7303. doi: DOI 10.1016/j.gca.2011.08.002.

848   Schulze, E.-D. *et al.* (1996) 'Rooting depth, water availability, and vegetation cover along an

849   aridity gradient in Patagonia', *Oecologia*, 108(3), pp. 503–511. doi: 10.1007/BF00333727.

850   Sime, L. C. *et al.* (2009) 'Evidence for warmer interglacials in East Antarctic ice cores.', *Nature*.

851   Nature Publishing Group, 462(7271), pp. 342–345. doi: 10.1038/nature08564.

852   Staccioli, G., Santoni, I. and Pizzo, B. (2014) 'Decay of fossil wood from kimberlite pipes of Lac

853   de Gras in the Canadian sub-Arctic area', *Annales de Paléontologie*, 100(1), pp. 87–94. doi:

854   10.1016/j.annpal.2013.11.004.

855   Sternberg, L. and Ellsworth, P. F. V. (2011) 'Divergent Biochemical Fractionation, Not

856   Convergent Temperature, Explains Cellulose Oxygen Isotope Enrichment across Latitudes',

857   *PLoS ONE*. Edited by H. Y. H. Chen, 6(11), p. e28040. doi: 10.1371/journal.pone.0028040.

858   Sternberg, L. S. L. *et al.* (2006) 'Variation in oxygen isotope fractionation during cellulose

859   synthesis: intramolecular and biosynthetic effects', *Plant, Cell & Environment*, 29(10), pp. 1881–

860   1889.

861   Sternberg, L. S. L. and DeNiro, M. J. (1983) 'Isotopic Composition of Cellulose from C3, C4, and

862   CAM Plants Growing Near One Another', *Science*, 220(4600), pp. 947–949. doi:

863   10.1126/science.220.4600.947.

864   Stokes, M. A. and Smiley, T. L. (1968) *An introduction to tree-ring dating*. The University of

865   Chicago Press, Chicago and London.

866   Stroeven, A. P., Prentice, M. L. and Kleman, J. (1996) 'On marine microfossil transport and

867   pathways in Antarctica during the late Neogene: Evidence from the Sirius Group at Mount

868   Fleming', *Geology*, 24(8), pp. 727–730. doi: 10.1130/0091-

869   7613(1996)024<0727:OMMTAP>2.3.CO;2.

870   Switsur, V. R. *et al.* (1995) 'Stable isotope studies in tree rings from oak—techniques and some

871   preliminary results', *Paläoklimaforschung*, 15, pp. 129–140.

872   Tipple, B. J., Meyers, S. R. and Pagani, M. (2010) 'Carbon isotope ratio of Cenozoic CO_2: A

873   comparative evaluation of available geochemical proxies', *Paleoceanography*, 25(3), p. n/a-n/a.

874   doi: 10.1029/2009PA001851.

875   Treydte, K. *et al.* (2014) 'Seasonal transfer of oxygen isotopes from precipitation and soil to the

876   tree ring: source water versus needle water enrichment.', *The New phytologist*, 202(3), pp. 772–

877   83. doi: 10.1111/nph.12741.

878   Treydte, K. S. *et al.* (2007) 'Signal strength and climate calibration of a European tree-ring

879   isotope network', *Geophysical Research Letters*, 34(24), p. L24302. doi:

880   10.1029/2007GL031106.

881   Warny, S. *et al.* (2009) 'Palynomorphs from a sediment core reveal a sudden remarkably warm

882   Antarctica during the middle Miocene', *Geology*, 37(10), pp. 955–958. doi: 10.1130/G30139A.1.

883   Webb, P.-N. and Harwood, D. M. (1993) 'Pliocene Fossil Nothofagus (Southern Beech) from

884   Antarctica: Phytogeography, Dispersal Strategies, and Survival in High Latitude Glacial-

885   Deglacial Environments', in *Forest Development in Cold Climates*, pp. 135–165.

886   Webb, P. N. *et al.* (1984) 'Geology Cenozoic marine sedimentation and ice-volume variation on

887   the East Antarctic craton Cenozoic marine sedimentation and ice-volume variation on the East

888   Antarctic craton', *Geology*, 12(5), pp. 287–291. doi: 10.1130/0091-7613(1984)12<287.

889   Webb, P. N. and Harwood, D. M. (1987) 'Terrestrial flora of the Sirius Formation: its significance

890   for Late Cenozoic glacial history', *Antarctic Journal of the United States*, 22(4), pp. 7–11.

891   Werner, C. and Gessler, A. (2011) 'Diel variations in the carbon isotope composition of respired

892   CO 2 and associated carbon sources: a review of dynamics and mechanisms', *Biogeosciences*,

893   8(9), pp. 2437–2459. doi: 10.5194/bg-8-2437-2011.

894   Werner, R. A. *et al.* (2011) 'Metabolic fluxes, carbon isotope fractionation and respiration -

895   lessons to be learned from plant biochemistry', *New Phytologist*, 191(1), pp. 10–15. doi:

896   10.1111/j.1469-8137.2011.03741.x.

897   West, A. G., Patrickson, S. J. and Ehleringer, J. R. (2006) 'Water extraction times for plant and

898   soil materials used in stable isotope analysis', *Rapid Communications in Mass Spectrometry*,

899   20(8), pp. 1317–1321. doi: 10.1002/rcm.2456.

900   Whitehead, J. M., Wotherspoon, S. and Bohaty, S. M. (2005) 'Minimal Antarctic sea ice during

901   the Pliocene', *Geology*, 33(2), pp. 137–140. doi: 10.1130/G21013.1.

902   Wieloch, T. *et al.* (2011) 'A novel device for batch-wise isolation of α-cellulose from small-

903   amount wholewood samples', *Dendrochronologia*, 29(2), pp. 115–117. doi:

904   10.1016/j.dendro.2010.08.008.

905   Wigley, T. M. L., Briffa, K. R. and Jones, P. D. (1984) 'On the Average Value of Correlated Time

906   Series, with Applications in Dendroclimatology and Hydrometeorology', *Journal of Climate and*

907   *Applied Meteorology*, 23(2), pp. 201–213. doi: 10.1175/1520-

908   0450(1984)023<0201:OTAVOC>2.0.CO;2.

909   Wolfe, A. P. *et al.* (2012) 'Pristine early eocene wood buried deeply in kimberlite from northern

910   Canada.', *PloS one*, 7(9), p. e45537. doi: 10.1371/journal.pone.0045537.

911   Woodcock, H. and Bradley, R. S. (1994) 'Salix arctica (pall.): its potential for

912   dendroclimatological studies in the High Arctic', *Dendrochronologia*, 12, pp. 11–22.

913   Xu, C. *et al.* (2016) 'Potential utility of tree ring δ 18 O series for reconstructing precipitation

914   records from the lower reaches of the Yangtze River, southeast China', *Journal of Geophysical*

915   *Research: Atmospheres*, 121(8), pp. 3954–3968. doi: 10.1002/2015JD023610.

916   Xu, C., Sano, M. and Nakatsuka, T. (2013) 'A 400-year record of hydroclimate variability and

917   local ENSO history in northern Southeast Asia inferred from tree-ring δ18O', *Palaeogeography,*

918   *Palaeoclimatology, Palaeoecology*, 386, pp. 588–598. doi: 10.1016/j.palaeo.2013.06.025.

919   Yakir, D. and Sternberg, L. S. L. (2000) 'The use of stable isotopes to study ecosystem gas

920   exchange', (January), pp. 297–311.

921   Yang, H. *et al.* (2009) 'Carbon and hydrogen isotope fractionation under continuous light:

922   implications for paleoenvironmental interpretations of the High Arctic during Paleogene

923   warming', *Oecologia*, 160(3), pp. 461–470. doi: 10.1007/s00442-009-1321-1.

924   Zhu, M. F. *et al.* (2012) 'Indo-Pacific Warm Pool convection and ENSO since 1867 derived from

925   Cambodian pine tree cellulose oxygen isotopes', *Journal of Geophysical Research-*

926   *Atmospheres*, 117. doi: Artn D11307 Doi 10.1029/2011jd017198.

927

928

Figure Captions

930

931 Figure 1: (**A**) Location of sampling sites on Isla Navarino in Tierra del Fuego, Chile. Yellow

932 triangle = marks the location of the GNIP station at Ushuaia; open circles = mark the tree ring

933 sampling sites. (**B**) Fossil wood location at Oliver Bluffs (black filled circle), Transantarctic

934 Mountains, Antarctica. White represents ice; grey shapes are Transantarctic Mountain outcrops.

935 (**C**) Photograph of exceptionally preserved fossil Nothofagus from Oliver Bluffs. (**D**) Scanning

936 Electron Microscope image of fossil *Nothofagus*, demonstrating excellent preservation of wood

937 fibres. (**E**) Prostrate *Nothofagus antarctica* from Isla Navarino.

938

939 Figure 2: (**A**) Standard deviation of $\delta^{13}C_{cell}$ (open circles) and $\delta^{18}O_{cell}$ (closed circles) with altitude

940 for Nothofagus from Isla Navarino, demonstrating a decrease in variability for $\delta^{18}O_{cell}$ for

941 prostrate trees. (**B**) Mean $\delta^{18}O_{cell}$ data for modern Nothofagus separated into arboreal and

942 prostrate form, and fossil Nothofagus. (**C**) As panel (**B**) but for $\delta^{13}C_{cell}$.

943

944 Figure 3: The relationship between modelled source water $\delta^{18}O$ and measured $\delta^{18}O_{source}$ water

945 for each site (from soils from each of the five sites (circles), roots from three sites (squares) and

946 Global Network of Isotopes in Precipitation $\delta^{18}O_{precip}$ (summer precipitation; diamond). Modelled

947 source water $\delta^{18}O$ was calculated from measured $\delta^{18}O_{cell}$ (modern *Nothofagus*) using the same

948 method as Anderson et al. (2002). Markers give the mean $\delta^{18}O$, y-error bars show the full

949 measured data range, x-errors show the range of modelled $\delta^{18}O_{source}$ if ε were varied within the

950 range given by Sternberg and DeNiro, (1983), and a 1:1 ratio is given by the dotted line for

951 comparison. Modelled data is calculated using RH = 0.7, f = 0.2.

952

953　Figure 4: Cellulose $\delta^{18}O$ from the Sirius Group fossil *Nothofagus*, with modelled $\delta^{18}O$ of

954　palaeo precipitation and modern Antarctic snow. Modern measurements from Masson-Delmotte

955　et al. (2008); data restricted to >75°S and below 700 masl. The median is given by the line, the

956　first and third quartiles by the box, and the whiskers denote the full range of data.