Response to:

***Interactive comment on** "Stable isotope paleoclimatology of the earliest Eocene using kimberlite-hosted mummified wood from the Canadian Subarctic" **by** B. A. Hook et al.*

Anonymous Referee #2

Received and published: 8 September 2015

Response by Benjamin A. Hook (corresponding author) on 10 September 2015

The comments made here by Referee #2 (*In Italics*) have led to improvements in the clarity and presentation of the Results and Discussion section, as well as details regarding the samples. Here I respond to each comment, explaining the changes that have been made in the text. Following this response is the previous response to Referee #1. Finally, the track-changes document prepared for the comments of Referee #1 is included, with these additional changes in response to Referee #2.

1. I would say that the parts "1. Introduction" and "2. Methods" in the manuscript are comparatively quite clear and well-organized. The part "3. Results and discussion", however, would terrify the readers. The authors should tease apart major points and re-organize this large block into fractions with sub-titles as they did in the parts "1. Introduction" and "2. Methods". This way it would be easier for readers to grasp the major points and their reasoning as well.

The "Results and discussion" section has been reorganized in a similar format as the "Methods" section, with subheadings for sections 3.1 Subannual-resolution study (P21 L379), 3.2 Annual-resolution study (P22 L426), 3.2.1 Tree-ring width and stable isotope correlations (P22 L427), 3.2.2 Oxygen isotope analysis (P24 L482), 3.2.3 Carbon isotope analysis (P26 L606), and 3.2.4 Dual-isotope analysis (P29 L750). Hopefully this will help to clarify the results for the reader.

2. Please give more details about the mummified wood and the tree rings the authors examined. Just one piece of wood? Or wood of many trees? The wood samples they examined were of one tree species or not? How did the authors select tree rings for their study? If tree rings were from different trees? Did different tree species respond the same way to the changing climatic factors?

More details on the mummified wood sampling have been added to the Methods and Results sections (2.1 and 3.2.1) to clarify these questions (P15 L202-224, and P22-23 L429-459, respectively). We sampled six pieces of Piceoxylon wood (same species), which appeared very similar in color, and tree-ring pattern. The samples were extracted during the course of diamond mining with heavy machinery by the Ekati Panda mining crew, and we were not present during excavation, so we are unsure of whether the samples originated from the same tree or not. However, some of the ring width correlations are unusually strong (R > 0.9), suggesting that the samples originated from the same tree at different places in the bole, and were later separated either during burial or removal from the ground. Three of the six samples were used for isotope analysis, creating an 86 y long master isotope chronology to compliment the 92 y long tree-ring width chronology.

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Hopefully these changes will make it clearer to the reader exactly which samples were used to construct these records.

3. Page 16280 Lines 15-24: logically it is not clear why PC1 corresponds with $\Sigma_{Z\text{-score}}$ and PC2 with $\Delta_{Z\text{-score}}$.

When conducting Principal Components Analysis (PCA) on $\delta^{13}C$ and $\delta^{18}O$, the first eigenvector (PC1) corresponded with the strongest relationship, which in this case is the positive correlation between $\delta^{13}C$ and $\delta^{18}O$. I found that the PC1 time series was nearly identical to a time series created by adding the normalized $\delta^{13}C$ and $\delta^{18}O$ datasets together ($\Sigma_{Z\text{-score}}$). Conversely, the second eigenvector is orthogonal to the first, and PC2 was found to be nearly identical to a time series created by subtracting the normalized $\delta^{13}C$ and $\delta^{18}O$ datasets ($\Delta_{Z\text{-score}}$). Adding two time series that are both influenced by a common climatic parameter should amplify that parameter. Conversely, subtracting those same series should minimize that factor, and amplify factors that the two time series do not share in common. For instance, if $\delta^{13}C$ and $\delta^{18}O$ are both influenced by relative humidity or stomatal conductance, summing their Z-scores should amplify that signal. Conversely, subtracting them should negate that signal and amplify other climatic factors such as sunlight availability (cloudiness) for $\delta^{13}C$, and source water $\delta^{18}O$ for $\delta^{18}O$ of cellulose. However, while the comparison of PC1 to $\Sigma_{Z\text{-score}}$, and PC2 to $\Delta_{Z\text{-score}}$, is quite interesting to me, it is not essential for the purposes of this article – the paleoclimatology of the early Eocene, and tends to confuse things for the reader more than is necessary. Therefore, I have decided to remove references to the PCA results, and focus instead only on $\Sigma_{Z\text{-score}}$ and $\Delta_{Z\text{-score}}$ parameters, because I feel these are more intuitively understandable. In the methods section (P20-21 L367-377), a few sentences explaining the similarity between PC1 and $\Sigma_{Z\text{-score}}$, and PC2 and $\Delta_{Z\text{-score}}$ were removed. Additionally, in the results section, references to PC1 and PC2 were removed, using instead the variables $\Sigma_{Z\text{-score}}$, and $\Delta_{Z\text{-score}}$ (P30-31 L778-812).

4. Page 16281 Line 4 and Line 14 and …: As the R values are quite small (below 0.5), this kind of relation can be termed as significant?

Despite the fact that the R-values for EPA3 vs EPA4 (0.38), and cross correlations of isotopes (0.27, 0.22, 0.23) are rather low, they were identified as significant if the p-values identified in the text were less than 0.05. Of course, "significance" is somewhat subjective to the interpretation of the reader, and varies by different scientific fields. In most ecological studies, p-values of less than 0.05 are commonly accepted. In the text, we have made changes to indicate that instances in which p-values < 0.05 but > 0.01 are termed as "marginally significant", and those values < 0.01 termed as "significant" (P23 L436, P23 L452-455). P-values and R-values are listed along with each analysis in the results. Additionally, we will include the source data along with the manuscript so that the reader may replicate the analyses.

5. Page 16282 Line 13: This passage is really confusing. Was the stomatal conductance an important factor or not?

If the first 4-8 tree rings were included, then the positive correlation *would* suggest that stomatal conductance was important. However, if these rings were omitted, there was no correlation, suggesting that stomatal conductance did not strongly affect the isotopes. Therefore, I have removed the statement "This suggests that stomatal conductance was an

important factor in the physiological functioning of these trees (Saurer et al., 1995)" for clarification of this section, because it does not seem that stomatal conductance was as important as other climatic factors (P22 L430).

Minors:

Page 16276 Line 26: delete one "delignification"

For some unknown reason, this error (writing delignification twice) does not exist in my original manuscript copy, only one "delignification" exists, so I have made no change here.

Page 16278 Line 16: define in the text what is "ebiochem" in the equation (3)

$\varepsilon_{biochem}$ is actually the same as ε_0 (the biochemical fractionation factor) described earlier in the text. I have therefore changed $\varepsilon_{biochem}$ to ε_0 in equation 3. (P17 L282)

Page 16278 Line22: define "MAT" when it first appears in the text

This has been done for this instance and for the first appearance in every other section (i.e. Introduction, Methods, etc.) in the text.

Page 16281 Line 16: d18C? Please change.

This has been changed to "$\delta^{13}C$".

Page 16285 Line 15: define "gs"

"as" has been changed to "because"

Additionally, I have made changes to the tree-ring numbering scheme, which was previously somewhat confusing because the text made references to the isotope chronology, which omitted the first 7 rings of the TRW chronology. In this version, all tree-ring (TR) numbers refer to the TRW chronology.

I would like to express my thanks to Reviewer 2 for their comments; which helped to improve the clarity of this manuscript.

All the best,
Benjamin A. Hook

Response to:

Interactive comment on "Stable isotope paleoclimatology of the earliest Eocene using kimberlite-hosted mummified wood from the Canadian Subarctic" *by* B. A. Hook et al.

Anonymous Referee #1

Received and published: 14 June 2015

Response by Benjamin A. Hook (corresponding author) on 16 July 2015

I would like to thank Referee #1 for their comments. I believe that they have led to improvements in this manuscript, especially in the carbon isotope section. Here, I respond to each comment, explaining the changes that have been made to the text.

1) What was the paleolatitude during the early Eocene with respect to modern latitude, and what are the paleoclimatic implications of a geographical transition?

This is a detail that had I meant to include, and mistakenly left it out, so I am glad that Referee brought it to my attention. The paleolatitude of the North American Arctic region has been estimated to be 62 ± 5 °N (McKenna 1980, Irving and Wynne 1991). Although considerable tectonic movement has altered its longitude, the paleolatitude is not much different than modern latitude (64° 42′ 49′′ N, 110° 37′ 10′′ W). Therefore, latitudinal influences on climate were not significantly different between the early Eocene and today. This point has been added to the introduction (page 16721, line 18) and in the site description in the methods section (page 16276, line 3).

2) What is the role of high pCO$_2$ on carbon isotope composition of the atmosphere and cellulose. Variables in isotopic discrimination models (c_i/c_a and ε_{pc}) were calculated in modern pCO$_2$, but how would higher pCO$_2$ influence these? Can free air carbon enrichment (FACE) studies give any insights into this issue?

This point addresses an important issue. One of the major difficulties of paleoclimatology in my opinion is the fact that when you have to analyze climates that are outside of the modern calibration range, extrapolation is necessary, which can sometimes lead to significant errors, if, for example relationships between variables are nonlinear. I looked more deeply into studies of plants growing in experimentally higher pCO$_2$ levels, including FACE studies (Battipaglia et al. 2013), the CLIMEX program (Beerling 1997), and controlled laboratory experiments using growth chambers (Lomax et al. 2012, Schubert and Jahren, 2012). One of the most intriguing studies regarding the relationship between pCO$_2$ and

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carbon isotope discrimination (Δ) is that of Schubert and Jahren (2012) who had unprecedentedly tight controls on hydrologic factors in the chambers, which allowed them to investigate this relationship. Whereas previous researchers had estimated linear relationships between Δ and $p\text{CO}_2$, but could not agree on the slope, Schubert and Jahren grew plants at a wide variety of $p\text{CO}_2$ levels, showing that the relationship is actually hyperbolic, such that it does not increase infinitely with higher $p\text{CO}_2$, but "levels off" or "flattens out" as it approaches a limit (28.26 ‰ in their study). These experiments were designed to elucidate the Δ vs. $p\text{CO}_2$ relationship, keeping the stomatal density (SD) constant. However, it is also known that during the geological past, SD has varied with $p\text{CO}_2$ level. This is the basis for the SD-$p\text{CO}_2$ proxy (Woodward 1986, 1987, Beerling 1997, Royer 2003, 2006, Beerling et al. 2009). Therefore, it seems likely that trees alter their SD (lower) during past greenhouse periods (high $p\text{CO}_2$). Particularly, the results of Beerling (1997) and recent experiments genetically altering SD and investigating isotopic fractionation variables (c_i/c_a, Δ) have been very enlightening (Doheny-Adams et al. 2012, Dow et al. 2014). Reducing SD in mutant Arabidopsis plants leads to reductions in c_i/c_a (Franks et al. 2015) but at higher $p\text{CO}_2$, c_i/c_a remains constant despite reduced SD (Beerling 1997). This mechanism shows how plants alter their SD to optimize water use efficiency in high $p\text{CO}_2$ environments. Additionally, Referee 1 commented that the ε_{pc} value, or the difference between $\delta^{13}C$ of bulk plant matter and cellulose, was measured in modern $p\text{CO}_2$ (ε_{pc} = 2 – 5 ‰; Barbour et al. 2002). Previously, we used the average εpc of modern wood (ε_{pc} = 3.5 ‰). However, Hook et al. (2015) recently measured ε_{pc} for mummified wood and cellulose (ε_{pc} = 3 ‰). Therefore, I have recalculated the affected data analysis accordingly using the value from Hook et al. (2015). I have added a few paragraphs explaining this issue in detail, in the methods section 2.3 Carbon Isotope Analysis (page 16279, line 8), wherein I add an additional $\delta^{13}C_{cellulose}$-$\delta^{13}C_{atm}$ transfer function by Lomax et al. (2012), take the arithmetic mean of transfer functions by Arens et al. (2000) and Lomax et al. (2012), as well as the commonly-used intrinsic water use efficiency (iWUE) equation (Farquhar et al. 1982, 1989). Additionally, I have added a few paragraphs to the results and discussion (page 16284, line 7), the conclusions (page 16289, line 2), the abstract (page 16270, line 17), table 3, and the highlights section, regarding this matter. I believe that my understanding of this issue has been improved, and that the manuscript is now better in this section as a result.

3) Explain the large difference in $\delta^{18}O$ isotopes during the subannually-sampled tree ring 42, in light of the fact that modern annual range is ~4‰.

Most modern studies of subannual $\delta^{18}O$ from tree rings find a smaller range around ~4 ‰. However, one of the tree rings analyzed here has a larger range of $\delta^{18}O$ (~5.5 ‰). This may be explained by a few different factors which are peculiar to the polar early Eocene climate. 1) increased amount effect from high rainfall potential (Dansgaard, 1964), 2) source water effect from freshwater Arctic Ocean (Brinkhuis et al., 2006), or 3) increased transpiration from polar forests with respect to today, recycling isotopically depleted water back into

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precipitation (Jasechko et al. 2013). An explanation of these factors has been added to the results and discussion section (page 16282, line 3).

4) Adjustments to font size and clarification of diagrams in figures 1 and 2.

These adjustments have been made to clarify the figures.

Additionally, I have made minor adjustments to the text for clarification, (page 16273, line 18 – "scenarios" to "situations" to reduce potential confusion with "scenarios" discussed later in carbon isotope discussion, the 3 scenarios discussed by Saurer et al. 2004 regarding c_i/c_a ratio in differing pCO$_2$. Also, I changed Hook et al., in review, to Hook et al., (2015) throughout, and updated the reference section with all of the new literature added.

Best,

Benjamin A. Hook

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Stable isotope paleoclimatology of the earliest Eocene using kimberlite-hosted mummified wood from the Canadian Subarctic

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Highlights

- High-resolution multi-proxy paleoclimatic study of early Eocene mummified wood

- Stable oxygen isotope mean annual temperature estimates were 11.4 °C

- Early Eocene intrinsic water use efficiency was > 2x modern levels

- Multidecadal oscillations (20–30 years per cycle) detected by dual-isotope analysis.

- Early Eocene oscillations similar to the modern Pacific Decadal Oscillation.

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Abstract

The recent discovery of well-preserved mummified wood buried within a subarctic kimberlite diamond mine prompted a paleoclimatic study of the early Eocene "hothouse" (*ca.* 53.3 Ma). At the time of kimberlite eruption, the Subarctic was warm and humid producing a temperate rainforest biome well north of the Arctic Circle. Previous studies have estimated mean annual temperatures in this region were 4—20 °C in the early Eocene, using a variety of proxies including leaf margin analysis, and stable isotopes ($\delta^{13}C$ and $\delta^{18}O$) of fossil cellulose. Here, we examine stable isotopes of tree-ring cellulose at subannual to annual scale resolution, using the oldest viable cellulose found to date. We use mechanistic models and transfer functions to estimate earliest Eocene temperatures using mummified cellulose, which was well preserved in the kimberlite. Multiple samples of *Piceoxylon* wood within the kimberlite were crossdated by tree-ring width. Multiple proxies are used in combination to tease apart likely environmental factors influencing the tree physiology and growth in the unique extinct ecosystem of the Polar rainforest. Calculations of interannual variation in temperature over a multidecadal time-slice in the early Eocene are presented, with a mean annual temperature (MAT) estimate of 11.4 °C (1 σ = 1.8 °C) based on $\delta^{18}O$, which is 16 °C warmer than the current MAT of the area (−4.6 °C). Early Eocene atmospheric $\delta^{13}C$ ($\delta^{13}C_{atm}$) estimates were −5.5 (± 0.7) ‰. Isotopic discrimination (Δ) and leaf intercellular pCO_2 ratio (c_i/c_a) were similar to modern values (Δ = 18.7 ± 0.8 ‰; c_i/c_a = 0.63 ± 0.03 %), but intrinsic water use efficiency (Early Eocene iWUE = 211 ± 20 μmol mol^{-1}) was over twice the level found in modern high-latitude trees. Dual-isotope spectral analysis suggests that multidecadal climate cycles somewhat similar to the modern Pacific Decadal Oscillation likely drove temperature and cloudiness trends on 20—30 year timescales, influencing photosynthetic productivity and tree growth patterns.

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1. Introduction

1.1 Warm subarctic climates of the earliest Eocene

If anthropogenic fossil fuel burning continues unabated, pCO$_2$ levels are expected to reach 855—

1130 ppmV by the end of the 21st century, leading to a 5.5 ± 0.6 °C temperature increase

globally with nearly twice as much warming in Arctic regions (IPCC, 2013). In this "worst-case"

climate change scenario, global temperatures will rapidly approach levels that have not existed

on Earth for over 50 million years, since the Eocene. Greenhouse climates of the earliest Eocene

were warm, with amplified warming at the poles (Greenwood and Wing, 1995), resulting from

high atmospheric pCO$_2$ levels (~680—3300 ppmV) (Schubert and Jahren, 2013). Permanent polar

ice caps did not exist; instead, vast temperate rainforests spanned the Arctic (Williams et al.,

2003), and Antarctica (Francis 1988; Francis and Poole, 2002; Ivany et al., 2011). The role that

these forests played in Eocene climates is unknown, because such rainforests do not currently

grow north of the Arctic Circle. Estimates of mean temperatures in the Eocene Arctic are much

warmer than today, but they range widely, from 4—20 °C, based on a variety of proxies [*e.g.,*

leaf physiognomy (Greenwood and Wing, 1995; Sunderlin et al., 2011), bacterial membrane

lipids (Weijers et al., 2007) oxygen isotope ratios in fossils of Eocene fauna (Fricke and Wing,

2004; Eberle et al., 2010), and oxygen isotopes of wood cellulose (Wolfe et al., 2012)]. Estimates

of climate variability would benefit modeling efforts of greenhouse climates (Huber and

Caballero, 2003) of past and future warm periods, but few studies have examined seasonal and

interannual fluctuations from the early Eocene (Eberle et al., 2010).

Recently, wood megafossils were discovered in kimberlite diamond mines in the Northwest

Territories of Canada (Wolfe et al., 2012). Paleolatitude of the study site during the early Eocene

81 [62 ± 5 °N (McKenna 1980, Irving and Wynne 1991)] was only a few degrees different than the

82 current location (64° 42′ 49′′ N, 110° 37′ 10′′ W). Therefore, latitudinal influences on climate

83 were similar between the early Eocene and today. These wood specimens are not petrified, but

84 mummified, many containing original woody material in a slightly altered state. A previous study

85 found that thermal alteration of this wood was low (< 60 °C) (Hook et al., 2015). FTIR spectra of

86 mummified *Piceoxylon* cellulose extracts matched those of modern cellulose. Preservation of

87 the wood was aided by their inclusion in adiabatically chilled post-eruptive kimberlite backfill

88 after eruption at *ca.* 53.3 Ma (Creaser et al., 2004). Samples of *Piceoxylon* Gothan 1905 wood

89 from the Ekati Panda pipe owned by Dominion Diamond Corp. contain α-cellulose matching the

90 composition of modern cellulose standards (Hook et al., 2015). Therefore, we used these

91 materials to investigate paleoclimates of the early Eocene, using a multi-proxy approach. By

92 gathering records of annual tree-ring width and stable isotopes of $\delta^{13}C$ and $\delta^{18}O$ from the same

93 tree rings, it is possible to glean more information than possible with a single proxy.

1.2 Stable isotopes in paleoenvironmental research

95 The ratio of $\delta^{18}O$ in precipitation (*i.e.,* source water – $\delta^{18}O_{sw}$) has a strong positive correlation

96 with temperature in terrestrial systems outside of the tropics: Cooler (warmer) climates at

97 higher latitudes and altitudes correspond with lower (higher) $\delta^{18}O_{sw}$. This has allowed

98 construction of isotopic maps that depict average $\delta^{18}O_{sw}$ across geographic regions (Bowen,

99 2010; Bowen and Revenaugh, 2003). Precipitation $\delta^{18}O_{sw}$ is influenced by temperature, but also

100 the location of evaporative sources, and continental rainout effects. Therefore, $\delta^{18}O_{sw}$ has been

101 used to reconstruct past temperatures from hydrologically sensitive archives, such as tree rings,

102 on an annual to subannual basis (DeNiro and Epstein, 1979; McCarroll and Loader, 2004; Roden

103 et al., 2009).

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108   After it was demonstrated that stable isotopes within tree rings could be used as an "isotopic

109   thermometer" of past climates (Libby and Pandolfi, 1974; Libby et al., 1976), there has been a

110   concerted effort to develop this proxy for the purposes of reconstructing temperatures before

111   the modern instrumental period. Mechanistic models have been developed which predict the

112   stable oxygen isotopic composition of α-cellulose ($\delta^{18}O_{cellulose}$) based on the isotopic ratio of

113   source water ($\delta^{18}O_{sw}$) received by the tree (Flanagan et al., 1991; Roden et al., 2000; Anderson

114   et al., 2002). These studies have found that in addition to $\delta^{18}O_{sw}$, factors that affect evaporative

115   enrichment of leaf water (*e.g.,* relative humidity – RH) also influence $\delta^{18}O_{cellulose}$. The problem

116   with using mechanistic models in paleoenvironmental research is that many of these

117   parameters (*e.g.*, early Eocene RH, leaf temperature) are unknown. However, one may estimate

118   a range of likely RH values and attain a range of likely temperature estimates based on the

119    $\delta^{18}O_{cellulose}$ (Wolfe et al., 2012; Csank et al., 2013). Another approach is a transfer function,

120   derived from plotting $\delta^{18}O_{cellulose}$ against $\delta^{18}O_{sw}$ from a number of samples and finding the best-

121   fit relationship between them (Ballantyne et al., 2006; Richter et al., 2008b; Csank et al., 2013).

122   Using this relationship, one may back-calculate an estimate of $\delta^{18}O_{sw}$ using $\delta^{18}O_{cellulose}$ of fossil

123   cellulose. Temperature may then be estimated from $\delta^{18}O_{sw}$ using a $\delta^{18}O$-temperature

124   relationship developed using isotope ratios of Eocene materials from different geographical

125   locations (Fricke and Wing, 2004).

126   Other factors may have affected $\delta^{18}O_{sw}$ besides temperature. The modern temperature–$\delta^{18}O_{sw}$

127   relationship (Dansgaard, 1964) is different than in the Eocene because polar ice caps and

128   glaciers are depleted in ^{18}O, and in the Eocene these ^{16}O-rich ice masses did not exist.

129   Additionally, in the Eocene "equable" climate, latitudinal temperature gradients were not as

130   steep as they are today, so condensation patterns may have been different (Greenwood and

131   Wing, 1995; Fricke and O'Neil, 1999). Plant transpiration sends isotopically light oxygen into the

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132   atmosphere, which may be used by other plants, thus decreasing $\delta^{18}O_{cellulose}$ more than would be

133   expected from temperature effects. The amount effect also lowers $\delta^{18}O_{sw}$ values through high

134   levels of precipitation. In modern climate, this factor is more prevalent in tropical areas near the

135   equator where heavy rainfall adds large amounts of ^{16}O, thus lowering the $\delta^{18}O_{sw}$ received by

136   plants.

137   Trees receive CO_2 through stomatal apertures in the leaves. During C_3 photosynthesis, trees

138   discriminate against CO_2 molecules containing ^{13}C resulting in a $\delta^{13}C$ depletion in plant matter

139   relative to ambient air. However, this effect is altered in two situations which increase $\delta^{13}C$ in

140   tree-ring records by reducing ^{13}C discrimination: (1) decreased relative humidity, leading to

141   decreased stomatal aperture and decreased availability of ^{12}C molecules during carbohydrate

142   fixation, and (2) increased photosynthetic rate as a result of increased sunlight availability. If a

143   tree is growing in an arid region, hydrologic factors (*e.g.,* vapor pressure deficit, relative

144   humidity, precipitation) are more likely to dominate the $\delta^{13}C$ signal because stomatal controls

145   over water loss also limit CO_2 intake, leading to higher $\delta^{13}C$ (Saurer et al., 1995; McCarroll and

146   Loader, 2004). When the tree receives more solar radiation the photosynthetic rate increases,

147   more CO_2 is required for glucose synthesis and ^{13}C discrimination is reduced, thus raising $\delta^{13}C$.

148   Clouds limit solar radiation, causing a drop in $\delta^{13}C$, along with reduced C sequestration and

149   photosynthetic assimilation (Alton, 2008). Therefore, records of $\delta^{13}C$ from *Pinus* trees growing

150   near the Arctic Circle in Fennoscandia show strong correlations with cloudiness, allowing $\delta^{13}C$

151   from tree-ring cellulose to be used as a proxy for cloud cover (Young et al., 2010, 2012;

152   Johnstone et al., 2013).

153   A common problem with studies of $\delta^{13}C$ in modern tree rings is related to the Suess effect,

154   which describes the modern day $\delta^{13}C$ decline due to the addition of fossil fuel CO_2 to the

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156   atmosphere (McCarroll and Loader, 2004). Because fossil fuels are derived from plant matter,

157   which discriminates against ^{13}C, the global average carbon isotope ratio ($\delta^{13}C_{atm}$) has dropped

158   from a pre-industrial average of −6.4 ‰ to the modern average around −8 ‰ (McCarroll and

159   Loader, 2004; McCarroll et al., 2009). In the early Eocene (*ca.* 53.3 Ma), $\delta^{13}C_{atm}$ was −5.7 ‰

160   based on isotopes of benthic foraminifera sampled from North Atlantic ocean sediments in

161   locations where surface waters sink to the ocean floor and are well mixed by the thermohaline

162   circulation (Tipple et al., 2010). Thus, δ^{13}C estimates from these benthic foraminifera record an

163   archive of surface water productivity levels, which are influenced by $\delta^{13}C_{atm}$ (Zachos et al., 2001).

164   Whereas $\delta^{13}C_{atm}$ varied on millennial timescales throughout the Cenozoic, it probably did not

165   vary significantly throughout the life of the trees in this study.

166   Analysis of δ^{18}O and δ^{13}C measured simultaneously from tree-ring cellulose ("dual-isotope"

167   analysis) may help constrain paleoclimatic signals better than a single isotopic ratio alone. As

168   some environmental factors influence both δ^{18}O and δ^{13}C through stomatal controls, and other

169   factors affect the isotopes independently, analyzing both isotopes together offers the possibility

170   of teasing apart environmental factors. Conceptual models of dual-isotope behavior in tree rings

171   in response to a range of environmental factors have been proposed (Scheidegger et al., 2000)

172   and tested (Roden and Farquhar, 2012), with theorized relationships holding true in some cases.

173   For example, factors affecting stomatal control influenced both δ^{18}O and δ^{13}C. Changing RH and

174   keeping all other variables fixed showed that δ^{18}O and δ^{13}C are indeed positively influenced by

175   RH, leading to the positive correlation between δ^{18}O and δ^{13}C observed in trees growing in arid

176   regions (Saurer et al., 1995, 1997). Low RH causes δ^{18}O to increase through evaporative loss of

177   ^{16}O molecules (H_2O molecules are smaller than CO_2 molecules, hence stomata have a reduced

178   effect compared to CO_2) (McCarroll and Loader, 2004). In water-stressed trees, leaf stomata

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179   have a strong control over the signals of both isotopes (Saurer et al., 1995); therefore dual-

180   isotope series show a positive correlation with each other through time (Saurer et al., 1997; Liu

181   et al., 2014). However, trees that grow in moist regions are typically not water-stressed, so

182   other factors not related to stomata are more likely to be dominant. For instance, low light

183   treatments affected $\delta^{13}C$ significantly, but not $\delta^{18}O$, indicating that $\delta^{13}C$ may be used as a proxy

184   for past light levels (Roden and Farquhar, 2012). In practice, records of cloud cover in

185   Fennoscandia match very closely to tree ring $\delta^{13}C$, leading to its use as a cloud cover proxy

186   (Young et al., 2010, 2012).

187   In this study, we measured tree-ring width and stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) at annual and

188   subannual resolution from tree-ring cellulose extracted from multiple samples of *Piceoxylon*

189   mummified wood. Our goal was to investigate seasonal, inter-annual, and possibly multidecadal

190   variability in tree growth and physiological functioning in this unique ancient ecosystem. The

191   extinct Polar Forest system is important to study, because it may allow improvements in

192   vegetation boundary conditions in paleoclimate and future climate models, which are currently

193   major sources of uncertainty (Huber and Caballero, 2011). For example, prodigious forest

194   growth in the Subarctic and Arctic may have had profound implications in positive warming

195   feedbacks, through changes in albedo and hydrologic regimes relative to today. Low albedo

196   would have caused direct warming, while greater transpiration by trees would have increased

197   water vapor in the Arctic atmosphere, which is a powerful greenhouse gas (Beerling and Franks,

198   2010; Jasechko et al., 2013). Therefore, Arctic temperature amplifications during equable

199   climates may be partially explained by transpiration-related increases in water vapor.

200   ## 2. Methods

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2.1 Tree-ring width measurement and cellulose extraction

Six samples of *Piceoxylon* Gothan 1905 mummified wood were excavated during diamond mining operations at Ekati Panda kimberlite mine. Paleolatitude of the site during the early Eocene was 62 ± 5 °N (McKenna, 1980; Irving and Wynne, 1991), which is similar to the modern location (64° 42' 49'' N, 110° 37' 10'' W), therefore the warm climates in this location are assumed not to be caused by lower latitude, but by other factors such as radiative forcing and climate feedbacks. The samples were surfaced, digitally scanned, and measured using a method developed specifically for mummified wood (Hook et al., 2013). Tree-ring series were crossdated using the skeleton plotting method (Stokes and Smiley, 1968), and the Dendrochronology Program Library in R (dplR) (Bunn, 2008, 2010). A floating chronology of tree ring width indices (RWI) (six samples, time series n = 92) was created using a 100-yr spline to remove the biological growth trend from the raw ring width series to focus on the underlying climate signal. Whereas RWI is a good parameter for general growth conditions, it responds to numerous climatic factors (*e.g.,* temperature, precipitation, sunlight). Tree ring width data was compared with isotope data from the same tree rings using cross-correlation analysis to test whether $\delta^{18}O$ or $\delta^{13}C$ had any significant associations with RWI in the same, or lagged, tree rings.

We dissected four individual tree rings (EPA3 rings 46-49) into subannual samples (ranging from n = 5 to n = 11 per tree ring) to capture the climatic signal from wood formed during the growing season. Along with this seasonal study we dissected entire tree rings from wood transects for an annual-resolution study (three crossdated mummified wood samples: EPA3, n = 42; EPA4, n = 54; EPA6, n = 43; master chronology time series 86 y long, see Supplemental Information). We selected these series due to strong correlations found in the tree-ring width series overlapping portions. Kimberlite minerals were removed from the outer bark edge of samples and cross-

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234   sections (3 cm thick) were cut. Then transects were cut from the cross-sections from pith to

235   bark, perpendicular to tree-ring boundaries. Transects were mechanically cleaned of kimberlite

236   minerals, and then dissected into annual or sub-annual samples using a reflected-light

237   microscope. Individual samples were placed in sterile glass vials and ground with a micro-pestle.

238   A Modified Brendel cellulose extraction method was used; heated acid hydrolysis (*via* strong

239   nitric/acetic acids) at 120 °C for 1 hour to ensure complete delignification. Following that, we

240   used a 2.5 % NaOH to remove hemicelluloses, which may have exchangeable oxygen atoms that

241   can be replaced by ambient (modern) oxygen and bias the signal (Brendel et al., 2000; Gaudinski

242   et al., 2005; Richter et al., 2008a; Hook et al., 2015). Stable isotope ratios were measured at the

243   Stable Isotope Laboratory at the University of Maryland. Cellulose was converted to carbon

244   monoxide CO at 1080 °C over glassy carbon within a stream of 99.99 % He. Sample gas was then

245   passed through traps for CO_2 and H_2O, and CO separated from N_2 by gas chromatography,

246   before isotopic analysis on Continuous-Flow Micromass/Elementar Isoprime coupled to a

247   Costech Analytical High Temperature Generator and Elemental Combustion System (Werner et

248   al., 1996). Carbon and oxygen isotopic data were corrected for runtime drift, amplitude

249   dependence and scaling using widely separated working cellulose isotopic standards calibrated

250   to international reference materials (Vienna Pee Dee Belemnite, VPDB for $\delta^{13}C$, and Standard

251   Mean Ocean Water, SMOW, for $\delta^{18}O$). The overall precisions for the corrected data, based on

252   replicate standard analyses, are 0.14 ‰ for $\delta^{13}C$ and 0.23 ‰ for $\delta^{18}O$.

253   # 2.2 Oxygen isotope analysis

254   To estimate early Eocene temperatures, the stable isotopic composition of $\delta^{18}O$ in tree ring

255   cellulose ($\delta^{18}O_{cellulose}$) was used to estimate $\delta^{18}O$ of source water ($\delta^{18}O_{sw}$) using mechanistic

262 models developed with modern plants (Roden et al., 2000). The Roden cellulose model uses a

263 leaf-water $\delta^{18}O_{leaf}$ model to predict from $\delta^{18}O$ of source water (Flanagan et al., 1991) using Eq. 1:

264 $\delta^{18}O_{wl} = \{(\alpha[\alpha_k * R_{wx}(e_i - e_a/e_i) + R_{wa}(e_a/e_i)]/0.0020052) - 1\} * 1000 ‰$ (1)

265 where R_{wx} and R_{wa} are the molar ratios of $^{18}O/^{16}O$ in leaf water, xylem water, and atmospheric

266 water, respectively, α is the fractionation factor for liquid-vapor equilibrium of water, which

267 depends on temperature (Majoube, 1971), α_k is the kinetic fractionation of water ($^{16}O/^{18}O$ =

268 1.0285), and e_i and e_a are the partial pressures of water vapor in leaf intercellular spaces and in

269 the atmosphere, respectively. Through a sensitivity analysis we found that the model was

270 insensitive to changes in temperature, so we used optimal leaf temperature during

271 photosynthesis (21.4 °C, Helliker and Richter, 2008) for calculation of α. Relative humidity (RH),

272 however, had a large influence on the outcome, so we used a range of likely RH values in a

273 temperate rainforest (64, 77, 83 %). The Roden et al. (2000) model uses the Flanagan et al.

274 (1991) leaf-water model to predict $\delta^{18}O_{cellulose}$ following Eq. 2:

275 $\delta^{18}O_{cellulose} = f_O * (\delta^{18}O_{wx} + \varepsilon_O) + (1 - f_O) * (\delta^{18}O_{wl} + \varepsilon_O)$ (2)

276 Here f_O is the fraction of carbon-bound oxygen that is subject to isotopic exchange (42 %), $\delta^{18}O_{wx}$

277 is the isotope ratio of xylem water and ε_O is the biochemical fractionation factor related to

278 conversion of sugar into cellulose (27 ‰). Xylem water is used as a close approximation to

279 source water, which is valid because no fractionation occurs between soil water and the

280 transference to xylem water (Barbour et al., 2002). Anderson et al., (2002) created a simplified

281 model that combined the Flanagan et al. (1991) leaf-water model with the Roden et al. (2000)

282 cellulose model, and reversed it to solve for $\delta^{18}O_{sw}$ using $\delta^{18}O_{cellulose}$ following Eq. 3:

283 $\delta^{18}O_{sw} \approx \delta^{18}O_{cellulose} - (1 - f) * (1 - h) + (\alpha + \alpha_k) - \varepsilon_O$ (3)

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285   Here f is a dampening factor related to isotopic fractionations between photosynthate and stem

286   water and h is relative humidity. In addition to these mechanistic models, we used several

287   transfer functions developed using modern tree-ring $\delta^{18}O_{cellulose}$ and its relationship to $\delta^{18}O_{sw}$

288   (Ballantyne et al., 2006, Richter et al., 2008b, Csank et al., 2013). A temperature–$\delta^{18}O_{sw}$

289   relationship developed for the Eocene was used to estimate the mean annual temperature

290   (MAT) based on $\delta^{18}O_{sw}$ (Fricke and Wing, 2004) (Table 1).

291   ## 2.3 Carbon isotope analysis

292   Isotopic discrimination against ^{13}C during photosynthesis has been modeled by Farquhar et al.

293   (1982, 1989) following Eq. 4:

294    $\Delta = a + (b - a)(c_i/c_a)$ (4)

295   where Δ is the discrimination against ^{13}C, a is the fractionation due to diffusion through air (4.4

296   ‰), b is the fractionation due to carboxylation by RuBisCO (27 - 30 ‰), c_i and c_a are the partial

297   pressures of CO_2 in the leaf intercellular spaces and atmosphere, respectively. Additionally, Δ

298   can be calculated by Eq. 5 (Farquhar et al., 1989):

299    $\Delta = (\delta^{13}C_{atm} - \delta^{13}C_p) / (1 + \delta^{13}C_p / 1000)$ (5)

300   where $\delta^{13}C_{atm}$ and $\delta^{13}C_p$ are the carbon isotope ratios of atmospheric CO_2 and bulk plant tissue,

301   respectively. To estimate $\delta^{13}C_{atm}$ from $\delta^{13}C_{cellulose}$ one may follow Eq. 6:

302    $\delta^{13}C_{atm} = \Delta + \delta^{13}C_{cellulose} - \varepsilon_{pc}$ (6)

303   where ε_{pc} is the carbon isotopic difference (‰) between cellulose ($\delta^{13}C_{cellulose}$) and bulk plant

304   matter ($\delta^{13}C_p$) (i.e., $\varepsilon_{pc} = \delta^{13}C_{cellulose} - \delta^{13}C_p$). Carbon isotope ratios of cellulose are typically 2—5

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313   ‰ higher (more enriched) than $\delta^{13}C$ of bulk plant tissue in the modern pCO_2 environment

314   (Barbour et al., 2002). Early Eocene-aged mummified *Piceoxylon* ε_{pc} values fell within the

315   modern ε_{pc} range, and are used in our calculations (ε_{pc} = 3 ‰; Hook et al., 2015). The parameters

316   a and b in the Farquhar et al., (1982) model (Eq. 4) are usually assumed to be constant, making

317   Δ dependent on the ratio of pCO_2 inside *v.* outside the leaf (c_i/c_a), which is unknown for the

318   Eocene. However, Δ could be estimated using $\delta^{13}C_{atm}$ from Eq. 6, then c_i/c_a by Eq. 4. The

319   relationship between carbon isotope ratios of plant matter ($\delta^{13}C_p$) and the atmosphere ($\delta^{13}C_{atm}$)

320   derived by Arens et al., (2000), following Eq. 7:

321   $\delta^{13}C_{atm} = (\delta^{13}C_{cellulose} + 18.72 - \varepsilon_{pc}) / 1.05$ (7)

322   Lomax et al. (2012) estimated the $\delta^{13}C_{atm}$–$\delta^{13}C_{cellulose}$ relationship using growth chamber

323   experiments, given by Eq. 8:

324   $\delta^{13}C_{atm} = (\delta^{13}C_{cellulose} + 15.71 - \varepsilon_{pc}) / 1.288$ (8)

325   As these equations are both based on empirical datasets that do not cover the full range of early

326   Eocene pCO_2, they may not represent the "true" relationship between $\delta^{13}C_{atm}$ and $\delta^{13}C_p$ at all c_a

327   levels. Therefore, we analyze them both as a possible range of values, and also take the

328   arithmetic mean of Eq.'s 7 and 8, which is given by Eq. 9:

329   $\delta^{13}C_{atm} = (\delta^{13}C_{cellulose} + 14.37 - \varepsilon_{pc}) / 1.1569$ (9)

330   To calculate c_i/c_a we substituted $\delta^{13}C_{atm}$ from Eq.'s 7, 8, and 9 into the $\delta^{13}C_{atm}$ term of Eq. 6 and

331   solved for Δ, then solved for c_i/c_a by rearranging Eq. 4, using Δ estimates and standard

332   fractionation constants (a = 4.4, b = 27; Farquhar et al., 1989). We then calculated intrinsic

333   water use efficiency (iWUE), a measure of carbon gained *vs.* water lost through stomatal

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346 apertures (Farquhar et al., 1982; 1989; Gagen et al., 2011) from Eq. 10, using c_a = 915 ppmV

347 (Schubert and Jahren, 2013).

348 $iWUE = (c_a - c_i) / 1.6$ $\qquad$ (10)

2.4 Dual-isotope analysis

350 Oxygen isotopes in cellulose are typically enriched by 20 to 30 ‰, whereas carbon isotopes are

351 depleted (−20 to −25 ‰ range). Therefore, to make the isotopes more comparable, both

352 datasets were normalized (mean = 0, variance = 1) and plotted together on one axis. The

353 normalized isotope time series were then summed (subtracted) to amplify (suppress) isotopic

354 variability common to both isotopes, and suppress (amplify) factors to which the isotopes do not

355 respond in a similar manner. For instance, changes in stomatal conductance (*e.g.,* due to

356 changes in relative humidity or drought) affect both isotopes, so if variance related to stomatal

357 conductance is large, the δ^{13}C and δ^{18}O time series should be positively correlated and vary in-

358 phase with each other (Saurer et al., 1997). Any variance in the dual-isotope series that is not

359 explained by this positive correlation is likely related to other factors. A factor that would likely

360 influence $\delta^{13}C_{cellulose}$ (but not $\delta^{18}O_{cellulose}$) is a reduction in light, possibly by cloud coverage

361 (Johnstone et al., 2013). On the other hand, $\delta^{18}O_{sw}$ would significantly affect $\delta^{18}O_{cellulose}$ (but not

362 $\delta^{13}C_{cellulose}$) (Ferrio and Voltas, 2005).

363 One way to amplify an environmental signal common to two proxies is addition. Adding the

364 normalized series together ($\Sigma_{Z\text{-score}}$) amplifies the in-phase components of the variance, and

365 suppresses the out-of-phase components. Conversely, subtracting the dual-isotope series from

366 each other ($\Delta_{Z\text{-score}}$) amplifies the out-of-phase components of the variance and suppresses the

367 in-phase components. Therefore, theoretically the $\Sigma_{Z\text{-score}}$ series should reflect variability

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373 associated with hydrologic factors related to stomatal conductance (e.g., relative humidity), and

374 the $\Delta_{Z\text{-score}}$ series should reflect variability related to sunlight and $\delta^{18}O$ of source water. Spectral

375 analysis was conducted [Multi-Taper Method, MTM (Mann and Lees, 1996); Singular Spectral

376 Analysis, SSA (Vautard and Ghil, 1989); kSpectra software] on the raw data, $\Sigma_{Z\text{-score}}$, and $\Delta_{Z\text{-score}}$

377 time series to examine the temporal power spectra.

3. Results and discussion

3.1 Subannual-resolution study

380 Days were long in the subarctic Eocene summer (~19 hr/d at summer solstace), allowing high

381 rates of photosynthesis, provided solar radiation was not obscured by clouds. In the subannual

382 study, the intra-annual series generally showed a rise and fall pattern throughout the growing

383 season, suggesting that this wood is of a persistent-leaved species (upper two graphs in Figure

384 1) (Barbour et al., 2001). Earlywood cellulose in deciduous species is isotopically enriched in $\delta^{13}C$

385 compared to persistent-leaves species, due to the use of carbohydrates stored in parenchyma

386 over the dormant season (Jahren and Sternberg, 2008). Changes in relative humidity (RH) may

387 explain a positive slope in a scatterplot of $\delta^{18}O$ and $\delta^{13}C$ (Roden and Farquhar, 2012);

388 theoretically, lowest RH (highest T) would be in midsummer when the continuous light regime is

389 near its peak (Figure 2). However, other factors besides RH probably affected the isotope signals

390 in most years not described by a simple rise and fall pattern along the RH slope. Tree ring (TR) 46

391 displayed a small range in $\delta^{18}O$ (1.7 ‰) and $\delta^{13}C$ (0.4 ‰) throughout the year possibly indicating

392 mild homogenous climate during that year (Figure 2). On the other hand, years with high solar

393 radiation but lower temperature variation may have raised the $\delta^{13}C$ without significantly altering

394 $\delta^{18}O$, as in the end of the season in TR 47.

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The range in $\delta^{18}O$ in ring 49 (5.6 ‰) was significantly larger than the average $\delta^{18}O$ range (< 4 ‰) in modern climates (Barbour et al., 2001). Possible reasons for the extreme seasonal range in TR 49 include an amount effect due to progressively larger amounts of late summer rains (Dansgaard, 1964), isotopically light source water recycled from the enclosed freshwater Arctic Ocean (Brinkhuis et al., 2006), or depleted water from forest transpiration (Jaseschko et al., 2013) reforming as precipitation. The first explanation (amount effect) is appealing due to the large tree-ring width seen in TR 49, which may have benefitted from long late-season rains, but all factors could have contributed to this large $\delta^{18}O$ range. Traumatic resin ducts were observed in TR 47 and 49, and these rings showed an irregular scatterplot pattern (Figure 2). Therefore, it is also possible that disturbance (*e.g.,* defoliation by insects) contributed to interruptions in these patterns. However, such disturbances are unlikely to substantially alter the climate signal on an annual basis, as modern trees do not show a strong isotopic response to disturbance from natural insect defoliation (Daux et al., 2011) or extreme experimental defoliation (Simard et al., 2012). Another factor in seasonal changes in $\delta^{13}C$ is an increase in $\delta^{13}C$ during peak growing season, when plants preferentially remove ^{12}C from the atmosphere (McCarroll and Loader, 2004).

3.2 Annual-resolution study

3.2.1 Tree-ring width and isotope correlations

Tree ring growth was prodigious in the earliest Eocene Subarctic [mean tree ring width for the six crossdated *Piceoxylon* samples ranged from 1.88—2.19 mm (σ range = 0.65—0.76, n = 92)] Ring width series in this study were sensitive enough for crossdating (mean sensitivity values = 0.20—0.36). The overlapping tree-ring width (TRW) sequences from the wood fragments were

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436   marginally positively correlated, supporting the idea that the trees were subjected to similar

437   climatic conditions (EPA3 *v.* EPA4, R = 0.38, p = 0.04, n = 30). In fact, some TRW series were so

438   similar that they may have originated from the same tree, and were separated during burial or

439   excavation (EPA4 *v.* EPA6, R = 0.90, p < 0.0001, n = 35). Because these six samples are all of the

440   same species, growing in the same area, we consider the master chronology produced here to

441   be a reflection of local climate and ontogenetic influences on the trees. TRW series were

442   detrended to remove ontogenetic (biological growth) patterns and focus on the climate signal.

443   However, many factors may influence TRW, so we measured stable isotopes from a subset of

444   tree rings to reconstruct specific climatic influences (e.g., temperature, solar radiation).

445   Stable isotopes were measured from three crossdated *Piceoxylon* samples (EPA3, EPA4, and

446   EPA6). Annual-resolution stable oxygen isotope (δ^{18}O) series were strongly correlated in both

447   overlapping sections (EPA3 *v.* EPA4, R = 0.78, p < 0.0001, n = 22; EPA4 *v.* EPA6, R = 0.85, p <

448   0.0001, n = 31) (lower two graphs in Figure 1). In one of the overlapping sections, δ^{13}C was

449   strongly correlated (EPA3 *v.* EPA4, R = 0.73, p < 0.0001, n = 22), but this was not the case in the

450   other overlapping section (EPA4 *v.* EPA6, R = 0.01, p = 0.97, n = 31). Both the RWI and δ^{18}O

451   records correlate strongly in this section so it is unknown why δ^{13}C does not. Cross-correlation

452   analysis of RWI and δ^{18}O isotope series found marginally significant relationships (p < 0.05)

453   when lagged by one or two years, suggesting that climatic conditions from the previous year or

454   two significantly influence tree-ring width [δ^{18}O lagged −1 year before RWI (R = 0.27, p = 0.02, n

455   = 84), δ^{18}O lagged −2 years before RWI (R = 0.22, p = 0.04, n = 83)]. Additionally, a marginally

456   positive correlation was found when δ^{13}C was lagged +2 with regard to RWI (R = 0.23, p = 0.04, n

457   = 83). This correlation may indicate that increased tree-ring growth is associated with increased

458   foliage production in the following years, thus leading to an increase in photosynthetic capacity

459   and hence an increase in δ^{13}C.

Annual-resolution master chronologies of both isotope series (δ^{13}C and δ^{18}O) were produced by taking the arithmetic mean of overlapping segments. The δ^{13}C and δ^{18}O master chronologies were positively correlated (Pearson's R = 0.36, P < 0.001, n = 86) (Figures 1 and 3). However, the first 4—8 tree rings were noticeably lower in δ^{13}C than the rest of the tree rings, presumably due to a juvenile effect in which growth conditions are different (*e.g.,* shadier) than mature trees. If these 4—8 rings are removed from analysis, the isotopes are no longer correlated (first four rings removed, Pearson's R = 0.17, P = 0.12, n = 82; first eight rings removed Pearson's R = 0.14, P = 0.22, n = 78). No correlation between the isotopes implies that stomatal conductance was less important than other climatic factors, suggesting that humid climates prevailed (Saurer et al., 1995). A previous study of middle Eocene (*ca.* 45 Ma) humidity found very high RH levels (80—100 %) by the end of the season in *Metasequoia* wood from high-Arctic Axel Heiberg Island (77 °N paleolatitude) (Jahren and Sternberg, 2008). Therefore, it is likely that high humidity with low variability existed at the Lac de Gras site during the early Eocene.

3.2.2 Oxygen isotope analysis

Using the annual-resolution δ^{18}O record, a range of temperature estimates was produced using the mechanistic models and transfer functions (Table 1). However, it is unknown which of these estimates is closest to actual Eocene temperatures. We estimated temperature based on different possible relative humidity (RH) levels (64, 77, 83 %), as in Wolfe et al. (2012) and Csank et al. (2013), and then calculated mean, standard deviation, 90 % confidence intervals, minimum and maximum of all models (Figures 4 and 5). Temperatures were generally warm according to this proxy record, staying above zero in the 90 % confidence interval; the range was 3.5—16.4 °C (n = 4), with a mean of 10.9 °C (1 σ = 3.0 °C) (black line in Figure 4). Warm month mean temperatures (WMMT) would therefore be at the higher end of this growing season range

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572   (~16.4 ± 3.0 °C), which is in agreement with published records of high Arctic seasonal

573   temperatures (19—20 °C, Eberle et al., 2010). Because tree-ring growth ceases during the

574   winter, cold month mean temperatures (CMMT) cannot be directly calculated with this archive.

575   However, we may estimate CMMT in comparison to mean annual temperature (MAT) using

576   independent estimates based on apatite of bowfin (*amiid*) fish that grow year-round suggesting

577   CMMT of 0—3.5 °C and an MAT of 8 °C (Eberle et al., 2010). In our annual study, the mean of all

578   of the methods (black line in Figure 5) ranged from 7.5—16.6 °C, with a mean of 11.4 °C (1 σ =

579   1.8 °C) (Table 2). This would suggest a CMMT of ~3.4—6.9 °C during the earliest Eocene based

580   on the difference between CMMT and MAT found by Eberle et al., (2010) applied to our MAT

581   estimate. The standard deviation of all methods was 4.1 °C, and the 90 % confidence interval

582   was 2.7 °C (Figure 5).

583   A mean temperature of 11.4 °C is close to other estimates of early Eocene MAT based on

584   independent proxies (*e.g.,* leaf margin analysis: 11—14 °C, Sunderlin et al., 2011). Some of the

585   highest MAT estimates produced (> 20 °C) match estimates of warmest mean temperatures for

586   the early Eocene (18—20 °C) (Weijers et al., 2007). Our MAT estimate is 2.4 °C higher than that

587   of Wolfe et al. (2012) (grand mean = 9 °C), but our mean estimate of 11.4 °C falls within the total

588   range of MAT estimates provided by that study (7—12 °C). Their study was conducted on $\delta^{18}O$

589   and δ^2H of cellulose from *Metasequoia* trees from the same kimberlite mine (n = 4). However,

590   bulk wood samples were taken in that study, precluding the possibility of examining distinct

591   years. We measured 141 individual tree rings from three crossdated tree-ring series spanning an

592   86-year-long period, and there were years in our record in which the MAT estimate was as low

593   as 9 °C as in Wolfe et al. (2012). It may be that the cellulose sampled in that study grew during

594   these years of slightly lower MAT, or that differences of 1—3 °C are not currently resolvable

595   using these proxies and the values are essentially equivalent.

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3.2.3 Carbon isotope analysis

The carbon isotopic composition of the atmosphere ($\delta^{13}C_{atm}$) changes slowly over million-year timescales (largely related to plate tectonic related forcing) (Zachos et al., 2001; Tipple et al., 2010). In the absence of a drastic release of atmospheric carbon such as the Paleocene-Eocene Thermal Maximum this value is assumed to be constant over an average tree lifespan (< 1000 yr). In this study, mean (± σ) $\delta^{13}C_{atm}$ estimates were −4.8 (± 0.7) ‰, −6.3 (± 0.6) ‰, and −5.5 (± 0.7) ‰ using Eq.'s 7, 8, and 9 respectively, based on mean (± σ) $\delta^{13}C_{cellulose}$ of −20.8 (± 0.8) ‰. This $\delta^{13}C_{atm}$ range matches the 90 % confidence interval of $\delta^{13}C_{atm}$ by Tipple et al. (2010) for the early Eocene (mean $\delta^{13}C_{atm}$ = −5.7 ‰; 90 % confidence interval: −4.8 to −6.3 ‰) based on isotopes of benthic foraminifera (Table 3). Solving for Δ in Eq. 6 gives 19.4 ‰ (from $\delta^{13}C_{atm}$ of Eq. 7), 17.9 ‰ (from $\delta^{13}C_{atm}$ of Eq. 8), and 18.7 ‰ (from $\delta^{13}C_{atm}$ of Eq. 9). Based on these Δ values, the c_i/c_a would be 0.66, 0.60, and 0.63, respectively. Assuming an early Eocene pCO_2 of 915 ppmV (Schubert and Jahren, 2013), these c_i/c_a values lead to intrinsic water use efficiency (iWUE) estimates of 192, 229, and 211 μmol mol⁻¹, respectively (Eq. 10) (Table 3). In modern climates, c_i/c_a may range from as low as 0.45 in *Picea crassifolia* Kom. growing in arid regions (Liu et al., 2007) to c_i/c_a values as high as 0.6 for *Picea glauca* (Moench) Voss. (Freeden and Sage, 1999) and 0.66 for *Picea abies* (L.) Karst (Wallin and Skärby, 1992) in greenhouse-grown *Pinus sylvestris* trees at ambient and increased pCO_2 and temperature (Beerling, 1997). These results suggest that the high pCO_2, high temperature conditions in the early Eocene subarctic, c_i/c_a values were similar to modern.

Saurer et al. (2004) proposed three possible scenarios regarding the behavior of plant fractionation (Δ) with increasing atmospheric pCO_2 (c_a): Scenario (1) leaf intercellular pCO_2 (c_i) remains constant with rising c_a, thus c_i/c_a decreases and internal water use efficiency (iWUE)

689 increases strongly; Scenario (2) c_i increases proportionally to c_a, causing c_i/c_a to remain relatively

690 constant and iWUE to increase; Scenario (3) c_i increases at about the same rate as c_a, and c_i/c_a

691 increases while iWUE remains constant. In free air carbon enrichment (FACE) plots, c_i/c_a tends to

692 decrease slightly (−0.02 to −0.08 %), but significantly, in high pCO$_2$ (~600 ppmV) with respect to

693 control plots (~400 ppmV), supporting Scenario (1) above (Battipaglia et al., 2013). However, the

694 opposite pattern is found in controlled growth chamber experiments (Lomax et al., 2012;

695 Schubert and Jahren, 2012). Using strict controls over hydrologic variables (*i.e.,* relative

696 humidity, soil water potential), Schubert and Jahren (2012) found that Δ is positively related to

697 pCO$_2$ by a hyperbolic function, such that Δ does not increase infinitely with increasing pCO$_2$ as

698 with a linear function, but flattens out as it approaches a limit of 28.26 ‰. This increase in Δ

699 may increase active carboxylation sites on RuBisCo, thus increasing c_i/c_a, which would support

700 Scenario (3) (Schubert and Jahren, 2012). However, these growth-chamber experiments were

701 designed to identify the relationship between Δ and pCO$_2$ at a constant stomatal density (SD =

702 number of stomata per unit area on the leaf). During the Eocene SD was lower than modern SD

703 in response to higher pCO$_2$, which would have affected gas exchange and water use efficiency

704 (Beerling et al., 2009).

705 Stomatal density or stomatal index (SI) of fossil leaves have long been used as paleo-pCO$_2$

706 proxies based on the observation that plants decrease SD and SI in high pCO$_2$ (Beerling et al.,

707 1998) and vice versa (Woodward, 1986; 1987) following a negative hyperbolic relationship that

708 flattens out at high pCO$_2$ levels (Royer, 2003; Beerling et al., 2009), mirroring the hyperbolic

709 relationship between Δ and pCO$_2$ (Schubert and Jahren, 2012). SD and SI display remarkable

710 phenotypic and genotypic plasticity to changing atmospheric pCO$_2$ over both short-term (*i.e.,*

711 hours to months) and long-term (*i.e.,* evolutionary) timescales (Beerling and Chaloner, 1993).

712 Reducing SD/SI during high pCO$_2$ maximizes efficiency in CO$_2$ uptake by leaf stomata, while

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713 minimizing water loss, thus resulting in iWUE over twice as much as modern iWUE in high-

714 latitude *Pinus* trees < 100 μmol mol^{-1}; Gagen et al. 2011). Greenhouse experiments with *Pinus*

715 *sylvestris* L. trees at elevated pCO$_2$ (560 ppmV) and temperature (+3 to 5 °C) show no change in

716 c_i/c_a despite reduced SD and increased iWUE (Beerling, 1997). Moreover, manipulations of SD

717 *via* epidermal patterning factor (EPF) genes in *Arabidopsis* mutants suggest that reduced

718 (increased) SD may lead to decreased (increased) transpiration and stomatal conductance (g_s),

719 along with increased (decreased) growth and iWUE (Doheny-Adams et al., 2012). Lower SD

720 causes reductions in c_i/c_a, which increases iWUE without changing photosynthetic capacity

721 (Franks et al., 2015). This optimizes operational stomatal conductance (g_{sop}) around a "sweet

722 spot" of 20 % maximum anatomical conductance (g_{smax}) (Dow et al., 2014). By operating at

723 around 20 % of g_{smax}, stomatal guard cells can be more responsive to rapid environmental

724 changes in RH or VPD. Therefore, the opposing hyperbolic curves (Δ vs. pCO$_2$, SD vs. pCO$_2$) may

725 balance out as a result of this phenotypic and genotypic plasticity, stabilizing Δ and c_i/c_a through

726 geologic time (Ehleringer and Cerling, 1995; Dawson et al., 2002), supporting Scenario (2) above

727 (Saurer et al., 2004).

728 In the modern climate, the Suess effect greatly alters δ^{13}C$_{atm}$, curving it unnaturally downward

729 starting with the industrial revolution, so tree ring records spanning this period must be

730 isotopically corrected (McCarroll et al., 2009). However, in the early Eocene average δ^{13}C$_{atm}$

731 levels were likely to be constant over the life of a tree in the absence of a hyperthermal event

732 (Zachos et al., 2001). Therefore, any shifts upward or downward around the mean δ^{13}C$_{cellulose}$ are

733 probably related to annual or seasonal changes in photosynthetic rate (A) or stomatal

734 conductance (g_s), both of which influence c_i/c_a. Photosynthesis would not have affected by high

735 pCO$_2$ under the continuous light of the polar summer (Beerling and Osborne, 2002), but may

736 have been affected by cloud-related reductions in sunlight (Young et a. 2010). We assume our

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745    $\delta^{13}C_{cellulose}$ record to be a qualitative proxy of sunlight/cloudiness, with the exception of a brief

746   period during the juvenile phase when trees must compete for light in the shaded understory,

747   leading to a juvenile effect in the early part of some $\delta^{13}C$ records (Gagen et al., 2007).

3.2.4 Dual-isotope analysis

749   Although precise quantitative estimates of sunlight cannot be made, analysis of both isotopes

750   simultaneously can aid in qualitative assessment of solar variability. When both isotope datasets

751   are normalized (Figure 6, top graph) and summed (Figure 6, middle graph), a signal related to RH

752   and vapor pressure deficit (VPD) should be amplified, because both isotopes are affected by

753   stomatal conductance (g_s) [low RH (high VPD) causes an increase in both $\delta^{18}O_{cellulose}$ and

754    $\delta^{13}C_{cellulose}$, leading to a positive correlation (Saurer et al., 1995)]. Conversely, when the dual

755   isotope data are normalized and subtracted, the remaining unexplained variance relating to

756   factors other than RH should be amplified (Figure 6, bottom graph). For $\delta^{18}O_{cellulose}$, $\delta^{18}O_{sw}$ is a

757   major factor (related to temperature of precipitation and precipitation sources), and for

758    $\delta^{13}C_{cellulose}$ cloudiness is the most likely controlling factor because clouds limit photosynthetic

759   rate. Modern trees growing near the Arctic Circle in Fennoscandia show high correlations

760   between annual records of stable carbon isotope ratios ($\delta^{13}C$) and records of cloud cover, where

761   the dominant factor in their $\delta^{13}C$ records is photosynthetic rate (Young et al., 2010, 2012). When

762   more sunlight is received, photosynthetic rate is increased, which reduces isotopic

763   discrimination and raises the $\delta^{13}C$ value. However, a converse relationship exists between

764   sunlight and temperature at different timescales. Proxy records suggest that at high frequency

765   (annual) timescales, sunlight and temperature are positively related (*i.e.,* sunny = warm, cloudy

766   = cool), but at low frequencies (multidecadal), they are negatively related (*i.e.,* cloudy = warm,

767   sunny = cool) (Young et al., 2012). This is somewhat counterintuitive but sustained, regional

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772 warmer temperatures cause an increase in evaporation and cloud cover, bringing latent heat to

773 northern latitudes through increased precipitation. Simultaneously, clouds cause short-term

774 local cooling by blocking solar radiation.

775 Spectral analysis of the normalized summed data ($\Sigma_{Z\text{-score}}$) shows a significant interannual-scale

776 pattern (2—3 ypc) (Figure 6, middle graph), whereas the normalized subtracted data ($\Delta_{Z\text{-score}}$)

777 shows multidecadal cyclicity (20—30 ypc) (Figure 6, bottom graph). This pattern is similar to

778 modes of the modern Pacific Decadal Oscillation (PDO) and Arctic Oscillation/North Atlantic

779 Oscillation (AO/NAO), which operate on multidecadal time-scales (Mantua et al., 1997, Young et

780 al., 2012). These modes are also teleconnected with ENSO cycles (2—7 ypc) in the modern

781 climate (Gershunov and Barnett, 1998). Temperature increases during positive phases of the

782 PDO contribute to greater evaporation, leading to enhanced cloud formation and precipitation

783 levels on a strongly bidecadal mode (Chiacchio et al., 2010). Sparse cloud cover may not

784 significantly block sunlight, as diffusion may redistribute it through the canopy (Reinhardt et al.,

785 2010; Urban et al., 2012). However, if cloud cover is very dense it may limit tree growth by

786 blocking photons necessary for photosynthesis (Ritchie, 2010). Heavy cloud cover has been

787 implicated in reduced photosynthetic rate of modern black spruce (*Picea mariana* (Mill.) Britton,

788 Sterns & Poggenburg) growing at subarctic treeline in Quebec, Canada (Vowinckel et al., 1975).

789 When dual-isotope analyses ($\Sigma_{Z\text{-score}}$ and $\Delta_{Z\text{-score}}$) were compared with RWI data, an apparent

790 positive association existed between $\Delta_{Z\text{-score}}$ and RWI at low frequencies. The middle portion

791 (*i.e.,* tree rings least likely affected by juvenile growth or diagenetic factors) of the 7-year

792 running mean data was strongly positively correlated (TR 34—89; R = 0.68, p < 0.0001, n = 55)

793 (Figure 7). This suggests that multidecadal climate fluctuations of temperature and precipitation

794 led to decades of increased tree growth during positive phases of the PDO-like pattern, and

30

807   decades of decreased growth during negative phases. No association was found between $\Sigma_{\text{Z-score}}$

808   and RWI records. If $\Sigma_{\text{Z-score}}$ is related to Eocene RH, sustained high humidity with low variability

809   may explain this non-association (Saurer et al., 1995).

810   In the early Eocene, subarctic trees may have been strongly dependent on both light and

811   precipitation, and therefore influenced by cloud coverage. Sewall and Sloan (2001) hypothesized

812   that in the Eocene, the lack of polar ice contributed to a stable positive Arctic Oscillation, rather

813   than the multidecadal dipole that currently exists. However, the RWI and isotope data

814   presented here suggest that PDO-like cyclicity operated in the early Eocene, possibly

815   contributing to AO teleconnections as it does today (Jia et al., 2009). Oceanic Rossby waves may

816   have set the timescale for multidecadal shifts in the position of the Aleutian low-pressure

817   system, which changes the trajectory of weather patterns (Gershunov and Barnett, 1998).

818   During positive PDO phases the position of the Aleutian low shifts southward, drawing in ENSO-

819   mediated tropical moisture and delivering it to the Subarctic (Figure 8). Another possibility for

820   the δ^{18}O variation is multidecadal shifts in source water location (*e.g.,* Pacific Ocean, Arctic

821   Ocean). In the early Eocene the Arctic Ocean was isolated from other oceans, with high

822   freshwater content from high precipitation (Brinkhuis et al., 2006). Thus, the Arctic Ocean

823   source water would have been depleted in δ^{18}O relative to Pacific Ocean source water.

824   Therefore, the trees in our study may have alternately received low-δ^{18}O from the Arctic, and

825   high-δ^{18}O from the Pacific shifting every 20—30 years.

826   Jahren and Sternberg (2002) suggested that meridional transport of precipitation northward

827   across the North American continent could have depleted the δ^{18}O of rainwater before reaching

828   their study site. However, such a strong southerly wind current system seems unlikely in the

829   Eocene, if the latitudinal temperature gradient was low (Greenwood and Wing, 1995), and given

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834 similar orbital variability (Laskar et al., 2011). However, if Eocene equatorial temperatures were

835 high (35—40 °C, Caballero and Huber, 2010) temperature gradients may have been stronger

836 than previously thought, leading to strong winds. Another possible explanation for the low $\delta^{18}O$

837 values of extreme northern polar forests in that study is that the source water was largely

838 recycled from depleted Arctic Ocean sources, or water transpired from trees (Jasechko et al.,

839 2013). Additionally, mineral contamination (*e.g.,* by iron oxides) may also cause negative $\delta^{18}O$

840 errors (Richter et al., 2008a). Paleoclimate models suggest that increases in atmospheric water

841 vapor due to an ice-free Arctic may have created conditions conducive to formation of a stable

842 Arctic cyclone, through which southern precipitation sources could not penetrate (Sewall and

843 Sloan, 2001). Our results suggest that if this stable Arctic cyclone existed then it probably still

844 had teleconnections with a PDO-like mechanism, causing the edge of the cyclone to shift

845 northward and southward on multidecadal timescales.

846 # 4. Conclusions

847 Multiple tree-ring based proxies were examined to study the climate of the early Eocene. The

848 material used was extremely well preserved *Piceoxylon* Gothan 1905 mummified wood found in

849 kimberlite diamond mines (*ca.* 53.3 Ma), which allowed geochemical investigations of primordial

850 cellulose. Stable isotope data ($\delta^{18}O$ and $\delta^{13}C$) were collected from subannually and annually

851 sampled increments along tree-ring chronologies. Mean annual temperatures (MAT) were

852 estimated to be 11.4 °C using $\delta^{18}O$ isotopes, taking the mean of a variety of commonly used

853 mechanistic models (Roden et al., 2000; Anderson et al., 2002) and transfer functions

854 (Ballantyne et al., 2006; Richter et al., 2008b; Csank et al., 2013) designed for estimating

855 temperature with wood cellulose. This value is in agreement with other studies using alternate

856 proxies (Greenwood and Wing, 1995; Sunderlin et al., 2011). The range is 7.5—16.6 °C, which is

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a 9 °C difference from warmest to coolest MAT. Seasonal climates were also investigated: mean annual range of temperature was 3.5—16.4 °C (n = 4), with a mean of 10.9 °C (1 σ = 3.0 °C). Warm month mean temperatures were ~16.4 ± 3.0 °C, but cold month mean temperatures could not be calculated with this archive, as the trees were dormant during winter when continuous darkness persisted. Our average estimate of δ^{13}C of Eocene atmosphere (−5.5 ± 0.7 ‰) based on transfer functions (Arens et al., 2000; Lomax et al., 2012) was in agreement with the estimate of Tipple et al. (2010) for *ca.* 53.3 Ma, who used independent proxy methods (*i.e.,* benthic foraminifera). Average estimates of δ^{13}C discrimination (Δ = 18.7 ± 0.8 ‰), and the ratio of leaf intercellular to atmospheric pCO_2 (c_i/c_a = 0.63 ± 0.03 %), were similar to those found in modern trees in ambient or elevated pCO_2 (Greenwood, 1997), supporting the hypothesis that c_i/c_a is stable through geologic time (Ehleringer and Cerling, 1995). Tree leaf stomatal density is reduced in high pCO_2, environments, causing intrinsic water use efficiency (iWUE) to be over twice as high as in modern trees. Assuming an early Eocene pCO_2 of 915 ppmV (Schubert and Jahren, 2013), iWUE = 211 ± 20 μmol mol^{-1}, which would explain the high levels of forest productivity observed in early Eocene polar forests (Williams, 2007). Dual-isotope analysis suggests that a strong interannual (2—3 ypc) signal related to stomatal functioning influenced both isotopes, as they are positively correlated ($\Sigma_{Z-score}$). However, if the first 4—8 tree rings representing juvenile growth are removed, the dual-isotopes are not correlated, suggesting that factors other than stomatal functioning are more important ($\Delta_{Z-score}$). Therefore, the most likely explanation for these patterns is that the dominant signal is related to multidecadal climate variability (*e.g.,* Pacific Decadal Oscillation, PDO) responsible for low-frequency shifts in δ^{18}O of source water, and δ^{13}C shifts related to cloudiness regimes on bidecadal (20—30 ypc) timescales.

References

894

895 Alton, P.B. Reduced carbon sequestration in terrestrial ecosystems under overcast skies
896   compared to clear skies. Agr Forest Meteorol 148, 1641—1653 (2008).

897 Anderson, W.T., Bernasconi, S.M., McKenzie, J.A., Saurer, M., Schweingruber, H.F. Model
898   evaluation for reconstructing the oxygen isotopic composition in precipitation from tree
899   ring cellulose over the last century. Chem Geol 182, 121—137 (2002).

900 Arens, N.C., Jahren, A.H., Amundson, R. Can C_3 plants faithfully record the carbon isotopic
901   composition of atmospheric carbon dioxide? Paleobiology 26, 137—164 (2000).

902 Ballantyne, A.P., Rybczynski, N., Baker, P.A., Harington, C.R., White, D. Pliocene Arctic
903   temperature contraints from the growth rings and isotopic composition of fossil larch.
904   Palaeogeogr Palaeocl 242, 188—200 (2006).

905 Barbour, M.M., Andrews, T.J., Farquhar, G.D. Correlations between oxygen isotope ratios of
906   wood constituents of *Quercus* and *Pinus* samples from around the world. Aust J Plant
907   Physiol 28, 335—348 (2001).

908 Barbour, M.M., Walcroft, A.S., Farquhar, G.D. Seasonal variation in $\delta^{13}C$ and $\delta^{18}O$ of cellulose
909   from growth rings of Pinus radiata. Plant Cell Environ 25, 1483—1499 (2002).

910 Beerling, D.L. Carbon isotope discrimination and stomatal responses of mature Pinus sylvestris L.
911   trees exposed in situ for three years to elevated CO_2 and temperature. Acta Ecol 18, 697-
912   712 (1997).

913 Beerling, D.J., Chaloner, W.G. Evolutionary responses of stomatal density to global CO_2 change.
914   Biol J Linn Soc 48, 343-353 (1993).

915 Beerling, D.J., Franks, P.J. The hidden cost of transpiration. Nature 464, 495—496 (2010).

916 Beerling, D.J., Osborne, C.P. Physiological Ecology of Mesozoic Polar Forests in a High CO_2
917   Environment. Ann Bot 89, 329-339 (2002).

918 Beerling, D.J., McElwain, J.C., Osborne, C.P. Stomatal responses of the 'living fossil' Ginkgo
919   biloba L. to changes in atmospheric CO_2 concentrations. J Ex Bot 49, 1603–1607 (1998).

920 Beerling, D.J., Fox, A., Anderson, C.W. Quantitative uncertainty analyses of ancient atmospheric
921   CO_2 estimates from fossil leaves. Am J Sci 309, 775-787 (2009).

922 Bowen, G.J. Isoscapes: Spatial pattern in isotopic biogeochemistry. Annu Rev Earth Pl Sc 38,
923   161—187 (2010).

34

924 Bowen, G.J., Revenaugh, J. Interpolating the isotopic composition of modern meteoric
925   precipitation. Water Resour Res 39, 1299 (2003).

926 Brendel, O., Ianetta, P.P.M., Stewart, D. A rapid and simple method to isolate pure alpha-
927   cellulose. Phytochem Analysis 11, 7—10 (2000).

928 Brinkhuis, H., Schouten, S., Collinson, M.E., Sluijs, A., Sinninghe Damsté, J.S., Dickens, G.R.,
929   Huber, M., Cronin, T.M., Onodera, J., Takahashi, K., Bujak, J.P., Stein, R., van der Burgh, J.,
930   Eldrett, J.S., Harding, I.C., Lotter, A.F., Sangiorgi, F., van Konijnenburg-van Cittert, H., de
931   Leeuw, J.W., Mattheissen, J., Backman, J., Moran, K., Clemens, S., Eynaud, F., Gattacceca,
932   J., Jakobsson, M., Jordan, R., Kaminski, M., King, J., Koc, N., Martinez, N.C., McInroy, D.,
933   Moore, Jr., T.C., O'Regan, M., Pälike, H., Rea, B., Rio, D., Sakamoto, T., Smith, D.C., St John,
934   K.E.K., Suto, I., Suzuki, N., Watanabe, M., Yamamoto, M. Episodic fresh surface waters in
935   the Eocene Arctic Ocean. Nature 441, 606—609 (2006).

936 Bunn, A.G. A dendrochronology program library in R (dplR). Dendrochronologia 26, 115—124
937   (2008).

938 Bunn, A.G. Statistical and visual crossdating in R using the dplR library. Dendrochronologia 28,
939   251—258 (2010).

940 Caballero, R., Huber, M. Spontaneous transition to superrotation in warm climates simulated by
941   CAM3. Geophys Res Lett 37, L11701 (2010).

942 Chiacchio, M., Ewen, T., Wild, M., Arabini, E. Influence of climate shifts on decadal variations of
943   surface solar radiation in Alaska. J Geophys Res 115, D00D21 (2010).

944 Creaser, R.A., Grütter, H., Carlson, J., Crawford, B. Macrocrystal phlogopite Rb-Sr dates for the
945   Ekati property kimberlites, Slave Province, Canada: Evidence for multiple intrusive
946   episodes in the Paleocene and Eocene. Lithos 76, 399—414 (2004).

947 Csank, A.Z., Fortier, D., Leavitt, S.W. Annually resolved temperature reconstructions from a late
948   Pliocene-early Pleistocene polar forest on Bylot Island, Canada. Palaeogeogr Palaeocl 369,
949   313—322 (2013).

950 Dansgaard, W. Stable isotopes in precipitation. Tellus 16, 368—436 (1964).

951 Daux, V., Edouard, J.L., Masson-Delmotte, V., Stievenard, M., Hoffmann, G., Pierre, M., Mestre,
952   O., Danis, P.A., Guibal, F. Can climate variations be inferred from tree-ring parameters and
953   stable isotopes from *Larix decidua*? Juvenile effects, budmoth outbreaks, and divergence
954   issue. Earth Planet Sc Lett 309, 221—233 (2011).

955 Dawson, T.E., Mambelli, S., Plamboeck, A.H., Templer, P.H., Tu, K.P. Stable isotopes in plant

956   ecology. Ann Rev Ecol Syst 33, 507-559 (2002).

957   DeNiro, M.J., Epstein, S. Relationship between the oxygen isotope ratios of terrestrial plant
958   cellulose, carbon dioxide, and water. Science 204, 51—53 (1979).

959   Doheny-Adams, T., Hunt, L., Franks, P.J., Beerling, D.J., Gray, J.E. Genetic manipulation of
960   stomatal density influences stomatal size, plant growth and tolerance to restricted water
961   supply across a growth carbon dioxide gradient. Phil Trans R Soc B 367, 547–555 (2012).

962   Dow, G.J., Bergmann, D.C., Berry, J.A. An integrated model of stomatal development and leaf
963   physiology. New Phytol 201, 1218–1226 (2014).

964   Eberle, J.J., Fricke, H.C., Humphrey, J.D., Hackett, L., Newbrey, M.G., Hutchison, J.H. Seasonal
965   variability in Arctic temperatures during early Eocene time. Earth Planet Sc Lett 296,
966   481—486 (2010).

967   Ehleringer, J.R., Cerling, T.E. Atmospheric CO_2 and the ratio of intercellular to ambient CO_2
968   concentrations in plants. Tree Physiol 15, 105-111 (1995).

969   Farquhar, G.D., O'Leary, M.H., Berry, J.A. On the relationship between carbon isotope
970   discrimination and the intercellular carbon dioxide in leaves. Aust J Plant Physiol 9, 121—
971   137 (1982).

972   Farquhar, G.D., Ehleringer, J.R., Hubick, K.T. Carbon isotope discrimination and photosynthesis.
973   Annu Rev Plant Phys 40, 503—537 (1989).

974   Farquhar, G.D., O'Leary, M.H., Berry, J.A. On the relationship between carbon isotope
975   discrimination and the intercellular carbon dioxide concentration in leaves. Aust J Plant
976   Physiol 9, 121-137 (1982).

977   Ferrio, J.P., Voltas, J. Carbon and oxygen isotope ratios in wood constituents of *Pinus halepensis*
978   as indicators of precipitation, temperature and vapour pressure deficit. Tellus 57B, 164—
979   173 (2005).

980   Flanagan, L.B., Comstock, J.P., Ehleringer, J.R. Comparison of Modeled and Observed
981   Environmental Influences on the Stable Oxygen and Hydrogen Isotope Composition of
982   Leaf Water in *Phaseolus vulgaris* L. Plant Physiol 96, 588—596 (1991).

983   Francis, J.E. A 50-Million-Year-Old Fossil Forest from Strathcona Fiord, Ellesmere Island, Arctic
984   Canada: Evidence for a Warm Polar Climate. Arctic 41, 314—318 (1988).

985   Francis, J.E., Poole, I. Cretaceous and early Tertiary climates of Antarctica: evidence from fossil
986   wood. Palaeogeogr Palaeocl 182, 47—64 (2002).

987   Franks, P.J., Doheny-Adams, T.W., Britton-Harper, Z.J., Gray, J.E. Increasing water-use efficiency

36

988   directly through genetic manipulation of stomatal density. New Phytol 207, 188–195

989   (2015).

990 Freeden, A.L., Sage, R.F. Temperature and humidity effects on branchlet gas-exchange in white

991   spruce: an explanation for the increase in transpiration with branchlet temperature. Trees

992   14, 161—168 (1999).

993 Fricke, H.C., O'Neil, J.R. The correlation between $^{18}O/^{16}O$ ratios of meteoric water and surface

994   temperature: its use in investigating terrestrial climate change over geologic time. Earth

995   Planet Sc Lett 170, 181—196 (1999).

996 Fricke, H.C., Wing, S.L. Oxygen isotope and paleobotanical estimates of temperature and $\delta^{18}O$-

997   latitude gradients over North America during the early Eocene. Am J Sci 304, 612—635

998   (2004).

999 Gagen, M., McCarroll, D., Loader, N.J., Robertson, I., Jalkanen, R., Anchukaitis, K.J. Exorcising the

1000   'segment length curse': summer temperature reconstruction since AD 1640 using non-

1001   detrended stable carbon isotope ratios from pine trees in northern Finland. Holocene 17,

1002   435—446 (2007).

1003 Gagen, M., Finsinger, W., Wagner-Cremer, F., McCarroll, D., Loader, N.J., Robertson, I., Jalkanen,

1004   R., Young, G., Kirchhefer, A. Evidence of changing intrinsic water-use efficiency under

1005   rising atmospheric CO_2 concentrations in Boreal Fennoscandia from subfossil leaves and

1006   tree ring $\delta^{13}C$ ratios. Global Change Biol 17, 1064–1072 (2011).

1007 Gaudinski, J.B., Dawson, T.E., Quideau, S., Schuur, E.A.G., Roden, J.S., Trumbore, S.E., Sandquist,

1008   D.R., Oh, S.-W., Wasylishen, R.E. Comparative analysis of cellulose preparation techniques

1009   for use with ^{13}C, ^{14}C, and ^{18}O isotopic measurements. Anal Chem 77, 7212—7224 (2005).

1010 Gershunov, A., Barnett, T.P. Interdecadal modulation of ENSO teleconnections. B Am Meteorol

1011   Soc 79, 2715—2725 (1998).

1012 Greenwood, D.R., Wing, S.L. Eocene continental climates and latitudinal temperature gradients.

1013   Geology 23, 1044—1048 (1995).

1014 Helliker, B.R., Richter, S.L. Subtropical to boreal convergence of tree-leaf temperatures. Nature

1015   454, 511—514 (2008).

1016 Hook, B., Halfar, J., Gedalof, Z., Bollmann, J. Controlled breaking of mummified wood for use in

1017   paleoenvironmental analysis. Tree-Ring Res 69, 87—92 (2013).

1018 Hook B., Halfar, J., Bollmann, J., Gedalof, Z., Rahman, M.A., Reyes, J., Schulze, D.J. Extraction of

1019 α-cellulose from mummified wood for stable isotopic analysis. Chem Geol 405 19–27

37

(2015).

Huber, M., Caballero, R. Eocene El Niño: Evidence for robust tropical dynamics in the "hothouse." Science 299, 877—881 (2003).

Huber, M., Caballero, R. The early Eocene equable climate problem revisited. Clim Past 7, 603—633 (2011).

Intergovernmental Panel on Climate Change (IPCC). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp. (2013).

Ivany, L.C., Brey, T., Huber, M., Buick, D.P., Schöne, B.R. El Niño in the Eocene greenhouse recorded by fossil bivalves and wood from Antarctica. Geophys Res Lett 38, L16709 (2011).

Jahren, A.H., Sternberg, L.S.L. Eocene meridional weather patterns reflected in the oxygen isotopes of Arctic fossil wood. GSA Today 12, 4—9 (2002).

Jahren, A.H., Sternberg, L.S.L. Annual patterns within tree rings of the Arctic middle Eocene (*ca.* 45 Ma): isotopic signatures of precipitation, relative humidity, and deciduousness. Geology 36, 99—102 (2008).

Jasechko, S., Sharp, Z.D., Gibson, J.J., Birks, J.S., Yi, Y., Fawcett, P.J. Terrestrial water fluxes dominated by transpiration. Nature 496, 347—350 (2013).

Jia, X., Lin, H., Derome, J. The influence of tropical Pacific forcing on the Arctic Oscillation. Clim Dynam 32, 495—509 (2009).

Johnstone, J.A., Roden, J.S., Dawson, T.E. Oxygen and carbon stable isotopes in coast redwood tree rings respond to spring and summer climate signals. J Geophys Res-Biogeo 118, 1438—1450 (2013).

Laskar, J., Fienga, A., Gastineau, M., Manche, H. La2010: A new orbital solution for the long term motion of the Earth. Astron Astrophys La2010 v4 (2011).

Libby, L.M., Pandolfi, L.J. Temperature dependence of isotope ratios in tree rings. PNAS 71, 2482—2486 (1974).

Libby, L.M., Pandolfi, L.J., Payton, P.H., Marshall, J.III., Becker, B., Giertz-Sienbenlist, V. Isotopic tree thermometers. Nature 261, 284—288 (1976).

Benjamin Hook 2015-7-13 10:06 AM
Deleted: Comparison of α-cellulose extraction techniques for use with kimberlite-hosted mummified wood. (In review at Chem Geol).

1055   Liu, X., Shao, X., Liang, E., Zhao, L., Chen, T., Qin, D., Ren, J. Species-dependent responses of

1056   juniper and spruce to increasing CO_2 concentration and to climate in semi-arid and arid

1057   areas of northwestern China. Plant Ecol 193, 195—209 (2007).

1058   Liu, X., An, W., Leavitt, S.W., Wang, W., Xu, G., Zeng, X., Qin, D. Recent strengthening of

1059   correlations between tree-ring $\delta^{13}C$ and $\delta^{18}O$ in mesic western China: Implications to

1060   climatic reconstruction and physiological responses. Global Planet Change 113, 23—33

1061   (2014).

1062   Lomax, B.H., Knight, C.A., Lake, J.A. An experimental evaluation of the use of C_3 $\delta^{13}C$ plant tissue

1063   as a proxy for the paleoatmospheric $\delta^{13}CO_2$ signature of air. Geochem Geophy Geosy 13,

1064   Q0AI03, (2012).

1065   Majoube, M. Fractionement en oxygéne-18 et en deutérium entre l'eau et sa vapeur. J Chem

1066   Phys 197, 1423—1426 (1971).

1067   Mann, M., Lees, J. Robust estimation of background noise and signal detection in climatic time

1068   series. Clim Change 33, 409—335 (1996).

1069   Mantua, N.J., Hare, S.R., Zhang, Y., Wallace, J.M., Francis, R.C. A Pacific interdecadal climate

1070   oscillation with impacts on salmon production. B Am Meteorol Soc 78, 1069—1079

1071   (1997).

1072   McCarroll, D., Loader, N.J. Stable isotopes in tree rings. Quat Sci Rev 23, 771—801 (2004).

1073   McCarroll, D., Gagen, M.H., Loader, N.J., Robertson, I., Anchukaitis, K., Los, S., Young, G.H.F.,

1074   Jalkanen, R., Kirchhefer, A., Waterhouse, J.S. Correction of tree ring stable carbon isotope

1075   chronologies for changes in carbon dioxide content of the atmosphere. Geochim

1076   Cosmochim Acta 73, 1539—1547 (2009).

1077   Reinhardt, K., Smith, W.K., Carter, G.A. Clouds and cloud immersion alter photosynthetic light

1078   quality in a temperate mountain cloud forest. Botany 88, 462—470 (2010).

1079   Richter, S.L., Johnson, A.H., Dranoff, M.M., LePage, B.A., Williams, C.J. Oxygen isotope ratios in

1080   fossil wood cellulose: Isotopic composition of Eocene- to Holocene-aged cellulose.

1081   Geochim Cosmochim Acta 72, 2744—2753 (2008a).

1082   Richter, S.L., Johnson, A.H., Dranoff, M.M., Taylor, K.D. Continental-scale patterns in modern

1083   wood cellulose $\delta^{18}O$: Implications for interpreting paleo-wood cellulose $\delta^{18}O$. Geochim

1084   Cosmochim Acta 72, 2735—2743 (2008b).

1085   Ritchie, R.J. Modelling photosynthetic photon flux density and maximum potential gross

1086   photosynthesis. Photosynthetica 48, 596—609 (2010).

39

1087 Roden, J.S., Lin, G., Ehleringer, J.R. A mechanistic model for interpretation of hydrogen and
1088   oxygen ratios in tree-ring cellulose. Geochim Cosmochim Acta 64, 21—35 (2000).
1089 Roden, J.S., Johnstone, J.A., Dawson, T.E. Intra-annual variation in the stable oxygen and carbon
1090   isotope ratios of cellulose in tree rings of coast redwood (*Sequoia sempervirens*).
1091   Holocene 19, 189—197 (2009).
1092 Roden, J.S., Farquhar, G.D. A controlled test of the dual-isotope approach for the interpretation
1093   of stable carbon and oxygen isotope ratio variation in tree rings. Tree Physiol 32, 490—
1094   503 (2012).
1095 Royer, D.L. Estimating latest Cretaceous and Tertiary atmospheric CO_2 from stomatal indices, *in*
1096   Wing, S.L., Gingerich, P.D., Schmitz, B., and Thomas, E., eds., Causes and Consequences of
1097   Globally Warm Climates in the Early Paleogene: Boulder, Colorado, Geological Society of
1098   America Special Paper 369, 79–93 (2003).
1099 Royer, D.L. CO_2-forced climate thresholds during the Phanerozoic. Geochim Cosmochim Acta 70,
1100   5665—5675 (2006).
1101 Saurer, M., Siegenthaler, U., Schweingruber, H.F. The climate-carbon isotope relationship in tree
1102   rings and the significance of site conditions. Tellus B, 47, 320—330 (1995).
1103 Saurer, M., Aellen, K., Siegwolf, R. Correlating $\delta^{13}C$ and $\delta^{18}O$ in cellulose of trees. Plant Cell
1104   Environ 20, 1543—1550 (1997).
1105 Saurer, M., Siegwolf, R.T.W., Schweingruber, F.H. Carbon isotope discrimination indicates
1106   improving water-use efficiency of trees in northern Eurasia over the last 100 years. Global
1107   Change Biol 10, 2109–2120 (2004).
1108 Scheidegger, Y., Saurer, M., Bahn, M., Siegwolf, R. Linking stable oxygen and carbon isotopes
1109   with stomatal conductance and photosynthetic capacity: A conceptual model. Oecologia
1110   125, 350—357 (2000).
1111 Schubert, B.A., Jahren, A.H. Reconciliation of marine and terrestrial carbon isotope excursions
1112   based on changing atmospheric CO_2 levels. Nat Commun 4, 1—6 (2013).
1113 Schubert, B.A., Jahren, A.H. The effect of atmospheric CO_2 concentration on carbon isotope
1114   fractionation in C_3 land plants. Geochim Cosmochim Acta 96, 29–43 (2012).
1115 Sewall, J.O., Sloan, L.C. Equable Paleogene climates: The result of a stable, positive Arctic
1116   Oscillation? Geophys Res Lett 28, 3693—3695 (2001).
1117 Simard, S., Morin, H., Krause, C., Buhay, W.M., Treydte, K. Tree-ring widths and isotopes of
1118   artificially defoliated balsam firs: A simulation of spruce budworm outbreaks in Eastern

40

1119   Canada. Environ Exp Bot 81, 44—54 (2012).

1120 Stokes, M.A., Smiley, T.L. An Introduction to Tree-Ring Dating, Univ. of Chicago Press, Chicago,
1121   Ill. 73 p. (1968).

1122 Sunderlin, D., Loope, G., Parker, N.E., Williams, C.J. Paleoclimatic and paleoecological
1123   implications of a Paleocene-Eocene fossil leaf assemblage, Chickaloon Formation, Alaska.
1124   Palaios 26, 335—345 (2011).

1125 Tipple, B.J., Meyers, S.R., Pagani, M. Carbon isotope ratio of Cenozoic CO_2: A comparative
1126   evaluation of available geochemical proxies. Paleoceanography 25, PA3202 (2010).

1127 Urban, O., Klem, K., Ač, A., Havránková, K., Holišová, P., Navrátil, M., Zitová, M., Kozlová, K.,
1128   Pokorný, R., Šprtová, M., Tomášková, I., Špunda, V., Grace, J. Impact of clear and cloudy
1129   sky conditions on the vertical distribution of photosynthetic CO_2 uptake within a spruce
1130   canopy. Funct Ecol 26, 46—55 (2012).

1131 Vautard, R., Ghil, M. Singular spectrum analysis in nonlinear dynamics, with applications to
1132   paleoclimatic time series. Physica D 32, 395—424 (1989).

1133 Vowinckel, T., Oechel, W.C., Boll, W.G. The effect of climate on the photosynthesis of Picea
1134   mariana at the subarctic tree line. 1. Field measurements. Can J Bot 53, 604—620 (1975).

1135 Wallin, G., Skärby, L. The influence of ozone on the stomatal and non-stomatal limitation of
1136   photosynthesis in Norway spruce, *Picea abies* (L.) Karst, exposed to soil moisture deficit.
1137   Trees 6, 128—136 (1992).

1138 Weijers, J.W.H., Schouten, S., Sluijs, A., Brinkhaus, H., Sinninghe Damsté, J.S. Warm arctic
1139   continents during the Palaeocene-Eocene thermal maximum. Earth Planet Sc Lett 261,
1140   230—238 (2007).

1141 Werner, R.A., Kornexl, B.E., Roßmann, A., Schmidt, H.L. On-line determination of $\delta^{18}O$-values of
1142   organic substances. Anal Chim Acta 319, 159—164 (1996).

1143 Williams, C.J. High-latitude Forest Structure: Methodological Considerations and Insights on
1144   Reconstructing High-latitude Fossil Forests. Bull Peabody Mus Nat Hist 48, 339–357
1145   (2007).

1146 Williams, C.J., Johnson, A.H., LePage, B.A., Vann, D.R., Sweda, T. Reconstruction of Tertiary
1147   *Metasequoia* forests. II. Structure, biomass, and productivity of Eocene floodplain forests
1148   in the Canadian Arctic. Paleobiology 29, 271—292 (2003).

1149 Wolfe, A.P., Csank, A.Z., Reyes, A.V., McKellar, R.C., Tappert, R., Muehlenbachs, K. Pristine Early
1150   Eocene wood buried deeply in kimberlite from northern Canada. PLoS ONE 7, e45537

41

1151   (2012).

1152   Woodward, F.I. Ecophysiological studies on the shrub Vaccinium myrtillus L. taken from a wide

1153   altitudinal range. Oecologia (Berlin) 70, 580-586 (1986).

1154   Woodward, F.I. Stomatal numbers are sensitive to increases in CO_2 from preindustrial levels.

1155   Nature 327, 617–618 (1987).

1156   Young, G.H.F., McCarroll, D., Loader, N.J., Gagen, M.H., Kirchhefer, A.J., Demmler, J.C. Changes

1157   in atmospheric circulation and the Arctic Oscillation preserved within a millennial length

1158   reconstruction of summer cloud cover from northern Fennoscandia. Clim Dynam 39,

1159   495—507 (2012).

1160   Young, G.H.F., McCarroll, D., Loader, N.J., Kirchhefer, A.J. A 500-year record of summer near-

1161   ground solar radiation from tree-ring stable carbon isotopes. Holocene 20, 315—324

1162   (2010).

1163   Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K. Trends, rhythms, and aberrations in global

1164   climate 65 Ma to present. Science 292, 686—693 (2001).

1165

Author Contributions

1167   B.A.H. designed study, collected and analyzed data, wrote manuscript, J.H. edited manuscript,

1168   Z.G. edited manuscript, J.B. edited manuscript, D.J.S. edited manuscript.

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Figure 1. Subannual and annual-resolution time series records of tree-ring cellulose δ^{18}O and δ^{13}C.
Subannual resolution a) δ^{18}O record, and b) δ^{13}C record, of four tree rings (TR 46—49). Lines above and below the measured values (bold center lines) show the analytical uncertainty (0.14 ‰ for δ^{13}C, 0.23 ‰ for δ^{18}O). Annual resolution c) δ^{18}O record, and d) δ^{13}C record (n = 86). Bold lines show mean isotope values of annual-resolution study, thin lines above and below mean values show minimum and maximum isotope values of successfully crossdated tree-ring transects (TR 28—63, 64—82).

Figure 2. Scatterplots of dual-isotope data for four tree rings (TR 46—49), showing trends of δ^{18}O and δ^{13}C within a growing season. Arrows point to the start of each numbered tree ring (earlywood), lines connect to consecutive samples (latewood) within each tree ring. Upper graph contains first two tree rings, and lower graph the third and fourth rings. Inset box in upper graph shows average low to high RH for *Pinus radiata* D. Don (after Roden and Farquhar, 2012). Low-to-high RH dual-isotope relationship: [δ^{13}C = 0.22 ∗ δ^{18}O − 31.31]. Scale is the same for inset graph, but actual values of Roden and Farquhar, (2012) (δ^{18}O low RH = 29.26 ‰, δ^{18}O high RH = 26.9 ‰; δ^{13}C low RH = −24.86 ‰, δ^{13}C high RH = −25.38 ‰) do not correspond with these axes.

Figure 3. Correlation analysis of dual-isotope annual dataset. δ^{18}O and δ^{13}C were significantly positively correlated (dashed trendline; Pearson's R = 0.36, P < 0.001, n = 86). However, if the first 4 – 8 "juvenile" tree rings (hollow circles) are removed from analysis, the remaining samples (filled circles) are not correlated (solid trendline; Pearson's R = 0.14, P = 0.22, n = 78).

Figure 4. Mean temperature (°C) of subannual data based on all δ^{18}O-temperature reconstructions. Mean of all reconstructions (black line) is bracketed by 90 % confidence interval (± 90 % ci, dark gray fill), one standard deviation (± 1 σ, medium gray fill), and minimum/maximum (± min/max, light gray fill). Freezing point is shown by dashed line.

Figure 5. Mean annual temperature (MAT °C) based on all δ^{18}O-temperature reconstructions. Mean of all reconstructions (black line) is bracketed by 90 % confidence interval (± 90 % ci, dark gray fill), one

1221 standard deviation (± 1 σ, medium gray fill), and minimum/maximum (± min/max, light gray fill)

1222 estimates.

1223 **Figure 6. Results of dual-isotope (δ^{18}O and δ^{13}C) analysis (n = 86).** *Upper panel:* Normalized δ^{18}O (δ^{18}O$_{Z\text{-}score}$,

1224 thin gray line) and δ^{13}C (δ^{13}C$_{Z\text{-}score}$ thin black line), and 7-yr triangular running mean δ^{18}O$_{Z\text{-}score}$ (bold

1225 gray line) and δ^{13}C$_{Z\text{-}score}$ (bold black line). *Center panel:* Sum of δ^{18}O$_{Z\text{-}score}$ and δ^{13}C$_{Z\text{-}score}$ ($\Sigma_{Z\text{-}score}$, thin gray

1226 line), and 7-yr triangular running mean (bold gray line). *Lower panel:* Difference of δ^{18}O$_{Z\text{-}score}$ minus δ^{13}C$_{Z\text{-}}$

1227 $_{score}$ ($\Delta_{Z\text{-}score}$, thin black line), and 7-yr triangular running mean (bold black line). Shaded regions in upper

1228 and lower panels highlight the bidecadal oscillations evident in the $\Delta_{Z\text{-}score}$ chronology in the lower panel.

1229 **Figure 7. Correspondence of *Piceoxylon* tree-ring width indices (RWI) and stable isotope chronologies.**

1230 (*Upper*) *Piceoxylon* RWI (n = 92, gray line) with 7-year triangular running mean (bold black line) to

1231 highlight low-frequency variability. (*Lower*) *Piceoxylon* isotope $\Delta_{Z\text{-}score}$ chronology (n = 86, gray line) with 7-

1232 year triangular running mean (bold black line) to highlight low-frequency variability. Here, grey boxes

1233 denote warmer and cloudier decades with above average tree ring growth. The first seven tree rings of

1234 the RWI record were not analyzed for stable isotopes, due to concerns about possible influences of

1235 juvenile tree growth on the isotope record. Question mark at the beginning of the TR record depicts

1236 uncertainty due to a possible juvenile growth signal.

1237 **Figure 8. Position and strength of Aleutian low-pressure system during positive and negative phases of**

1238 **the PDO in relation to study site.** Hypothesized stable Arctic Oscillation during the Eocene depicted by

1239 grey arc in upper right corner (*see Sewall and Sloan 2001). 1000 mb sea level pressure (SLP) contours

1240 shown for negative PDO (blue shaded area) and positive PDO (red shaded area). Weather patterns are

1241 altered according to these changes in SLP (blue arrow – negative PDO, red arrow – positive PDO), thus

1242 altering the distribution of precipitation across North America. Positions of 1000 mb contours of Aleutian

1243 low after NOAA-CIRES/Climate Diagnostics Center (Jan-Mar sea level pressure (mb) composite for

1244 negative PDO 1988, 1999; for positive PDO 1983, 1987, 1992, 1998).

1257 **Table 1. Summary of equations used in oxygen isotope temperature reconstruction.** Mechanistic models
1258 and transfer functions used to predict $\delta^{18}O_{sw}$ from $\delta^{18}O_{cellulose}$, and a temperature–$\delta^{18}O_{sw}$ relationship
1259 developed for the Eocene (Fricke and Wing, 2004). Shown are each equation and the reference on which
1260 it is based.

Type of analysis	Used to calculate	Reference
Mechanistic models		
$\delta^{18}O_{wl} = \{(\alpha[\alpha_k * R_{wx}(e_i - e_a/e_i) + R_{wa}(e_a/e_i)] / 0.0020052) - 1\} * 1000$	$\delta^{18}O_{wl}$	Flanagan et al., 1991
$\delta^{18}O_{cellulose} = f_O * (\delta^{18}O_{wx} + \varepsilon_O) + (1 - f_O) * (\delta^{18}O_{wl} + \varepsilon_O)$	$\delta^{18}O_{wx}$	Roden et al., 2000[#]
$\delta^{18}O_{sw} \approx \delta^{18}O_{cellulose} - (1 - f) * (1 - h) + (\alpha + \alpha_k) - \varepsilon_{biochem}$	$\delta^{18}O_{sw}$	Anderson et al., 2002
Transfer functions		
$\delta^{18}O_{sw} = 312.75 * e^{(-0.13 * d18Ocellulose)}$	$\delta^{18}O_{sw}$	Ballantyne et al., 2006
$\delta^{18}O_{sw} = (\delta^{18}O_{cellulose} - 35.11) / 0.59$	$\delta^{18}O_{sw}$	Richter et al., 2008b*
$\delta^{18}O_{sw} = (\delta^{18}O_{cellulose} - 33.2045) / 0.6109$	$\delta^{18}O_{sw}$	Csank et al., 2013*
$\delta^{18}O_{sw} = -0.01T^2 + T - 22.91$	$T (°C)$	Fricke and Wing, 2004**

[#]*Equation solved for $\delta^{18}O_{wx}$, which is used as a surrogate for $\delta^{18}O_{sw}$*
Linear transfer functions estimating $\delta^{18}O_{cellulose}$ were solved for $\delta^{18}O_{sw}$ as shown here.
***A fourth-order polynomial, based on the Fricke and Wing (2004) polynomial shown here, was used to
estimate T (°C) based on the different $\delta^{18}O_{sw}$ estimates from mechanistic models and transfer functions:**
$T (°C) = (0.000005 * \delta^{18}O_{sw}^4) + (0.0007 * \delta^{18}O_{sw}^3) + (0.0436 * \delta^{18}O_{sw}^2) + (2.1153 * \delta^{18}O_{sw}) + 32.697$*

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1262 **Table 2. Early Eocene Mean Annual Temperature (MAT) estimates based on $\delta^{18}O$ of *Piceoxylon***
1263 **cellulose.** Several methods of temperature estimation in the literature were used, including mechanistic
1264 models (Roden et al., 2000; Anderson et al., 2002) and transfer functions (Csank et al., 2013; Richter et al.,
1265 2008b; Ballantyne et al., 2006) that predict $\delta^{18}O_{sw}$ from $\delta^{18}O_{cellulose}$. MAT was derived from $\delta^{18}O_{sw}$ using a
1266 $\delta^{18}O_{sw}$–temperature relationship developed for the Eocene (Fricke and Wing, 2004). Shown are references
1267 for model/function, relative humidity level (*for mechanistic models*), range (min—max) of MAT (°C), and
1268 mean (standard deviation) of MAT (°C) in chronology.

Reference	Relative Humidity	Range MAT (°C)	Mean (sd) MAT (°C)
Mechanistic Models			
Roden et al., 2000	64 %	1—12.6	5.9 (2.3)
	77 %	4.6—17.4	10.0 (2.6)
	83 %	6.1—19.5	11.7 (2.7)
Anderson et al., 2002	64 %	10.6—16.3	13.1 (1.2)
	77 %	13.3—19.5	16.0 (1.3)
	83 %	15.3—21.9	18.2 (1.3)
Transfer Functions			
Csank et al., 2013		6.5—15.4	10.3 (1.8)
Richter et al., 2008b		2.4—10.5	5.9 (1.7)
Ballantyne et al., 2006		7.7—16.4	11.9 (1.8)
Mean of all methods		*7.5—16.6*	*11.4 (1.8)*

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Table 3. Early Eocene $\delta^{13}C_{atm}$, Δ, c_i/c_a, and intrinsic water use efficiency (iWUE) estimates. $\delta^{13}C_{atm}$ (‰) results of Tipple et al. (2010) (mean and 90 % confidence interval bounds), are compared with results from this study: Equation used to calculate $\delta^{13}C_{atm}$ (‰), along with estimates of Δ (‰), c_i/c_a (%), and iWUE (µmol mol^{-1}). Average early Eocene pCO$_2$ of 915 ppmV was used (Schubert and Jahren, 2013).

Tipple et al. (2010)		This Study				
Bounds	$\delta^{13}C_{atm}$	Equation	$\delta^{13}C_{atm}$	Δ	c_i/c_a	iWUE
Lower 90 %	−4.8	Eq. 7	−4.8	19.4	0.66	192
Upper 90 %	−6.3	Eq. 8	−6.3	17.9	0.60	229
Mean	−5.7	Eq. 9	−5.5	18.7	0.63	211