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)

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 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 pCO_2, but could not agree on the slope, Schubert and Jahren grew plants at a wide variety of pCO_2 levels, showing that the relationship is actually hyperbolic, such that it does not increase infinitely with higher pCO_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. pCO_2 relationship, keeping the stomatal density (SD) constant. However, it is also known that during the geological past, SD has varied with pCO_2 level. This is the basis for the SD-pCO_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 pCO_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 pCO_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 pCO_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 pCO_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 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

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-day Pacific Decadal Oscillation.

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1. 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 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.

2. Introduction

2.1 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

[62 ± 5 °N (McKenna 1980, Irving and Wynne 1991)] was only a few degrees different than the current location (64° 42′ 49′′ N, 110° 37′ 10′′ W). Therefore, latitudinal influences on climate were similar between the early Eocene and today. These wood specimens are not petrified, but mummified, many containing original woody material in a slightly altered state. A previous study found that thermal alteration of this wood was low (< 60 °C) (Hook et al., 2015). FTIR spectra of mummified *Piceoxylon* cellulose extracts matched those of modern cellulose. Preservation of the wood was aided by their inclusion in adiabatically chilled post-eruptive kimberlite backfill after eruption at *ca.* 53.3 Ma (Creaser et al., 2004). Samples of *Piceoxylon* Gothan 1905 wood from the Ekati Panda pipe owned by Dominion Diamond Corp. contain α-cellulose matching the composition of modern cellulose standards (Hook et al., 2015). Therefore, we used these materials to investigate paleoclimates of the early Eocene, using a multi-proxy approach. By gathering records of annual tree-ring width and stable isotopes of $\delta^{13}C$ and $\delta^{18}O$ from the same tree rings, it is possible to glean more information than possible with a single proxy.

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2.2 Stable isotopes in paleoenvironmental research

The ratio of $\delta^{18}O$ in precipitation (*i.e.,* source water – $\delta^{18}O_{sw}$) has a strong positive correlation with temperature in terrestrial systems outside of the tropics: Cooler (warmer) climates at higher latitudes and altitudes correspond with lower (higher) $\delta^{18}O_{sw}$. This has allowed construction of isotopic maps that depict average $\delta^{18}O_{sw}$ across geographic regions (Bowen, 2010; Bowen and Revenaugh, 2003). Precipitation $\delta^{18}O_{sw}$ is influenced by temperature, but also the location of evaporative sources, and continental rainout effects. Therefore, $\delta^{18}O_{sw}$ has been used to reconstruct past temperatures from hydrologically sensitive archives, such as tree rings, on an annual to subannual basis (DeNiro and Epstein, 1979; McCarroll and Loader, 2004; Roden et al., 2009).

After it was demonstrated that stable isotopes within tree rings could be used as an "isotopic thermometer" of past climates (Libby and Pandolfi, 1974; Libby et al., 1976), there has been a concerted effort to develop this proxy for the purposes of reconstructing temperatures before the modern instrumental period. Mechanistic models have been developed which predict the stable oxygen isotopic composition of α-cellulose ($\delta^{18}O_{cellulose}$) based on the isotopic ratio of source water ($\delta^{18}O_{sw}$) received by the tree (Flanagan et al., 1991; Roden et al., 2000; Anderson et al., 2002). These studies have found that in addition to $\delta^{18}O_{sw}$, factors that affect evaporative enrichment of leaf water (*e.g.,* relative humidity – RH) also influence $\delta^{18}O_{cellulose}$. The problem with using mechanistic models in paleoenvironmental research is that many of these parameters (*e.g.*, early Eocene RH, leaf temperature) are unknown. However, one may estimate a range of likely RH values and attain a range of likely temperature estimates based on the $\delta^{18}O_{cellulose}$ (Wolfe et al., 2012; Csank et al., 2013). Another approach is a transfer function, derived from plotting $\delta^{18}O_{cellulose}$ against $\delta^{18}O_{sw}$ from a number of samples and finding the best-fit relationship between them (Ballantyne et al., 2006; Richter et al., 2008b; Csank et al., 2013). Using this relationship, one may back-calculate an estimate of $\delta^{18}O_{sw}$ using $\delta^{18}O_{cellulose}$ of fossil cellulose. Temperature may then be estimated from $\delta^{18}O_{sw}$ using a $\delta^{18}O$-temperature relationship developed using isotope ratios of Eocene materials from different geographical locations (Fricke and Wing, 2004).

Other factors may have affected $\delta^{18}O_{sw}$ besides temperature. The modern temperature–$\delta^{18}O_{sw}$ relationship (Dansgaard, 1964) is different than in the Eocene because polar ice caps and glaciers are depleted in ^{18}O, and in the Eocene these ^{16}O-rich ice masses did not exist. Additionally, in the Eocene "equable" climate, latitudinal temperature gradients were not as steep as they are today, so condensation patterns may have been different (Greenwood and Wing, 1995; Fricke and O'Neil, 1999). Plant transpiration sends isotopically light oxygen into the atmosphere, which may be used by other plants, thus decreasing $\delta^{18}O_{cellulose}$ more than would be expected from temperature effects. The amount effect also lowers $\delta^{18}O_{sw}$ values through high levels of precipitation. In modern climate, this factor is more prevalent in tropical areas near the equator where heavy rainfall adds large amounts of ^{16}O, thus lowering the $\delta^{18}O_{sw}$ received by plants.

Trees receive CO_2 through stomatal apertures in the leaves. During C_3 photosynthesis, trees discriminate against CO_2 molecules containing ^{13}C resulting in a $\delta^{13}C$ depletion in plant matter relative to ambient air. However, this effect is altered in two situations which increase $\delta^{13}C$ in tree-ring records by reducing ^{13}C discrimination: (1) decreased relative humidity, leading to decreased stomatal aperture and decreased availability of ^{12}C molecules during carbohydrate fixation, and (2) increased photosynthetic rate as a result of increased sunlight availability. If a tree is growing in an arid region, hydrologic factors (*e.g.,* vapor pressure deficit, relative humidity, precipitation) are more likely to dominate the $\delta^{13}C$ signal because stomatal controls over water loss also limit CO_2 intake, leading to higher $\delta^{13}C$ (Saurer et al., 1995; McCarroll and Loader, 2004). When the tree receives more solar radiation the photosynthetic rate increases, more CO_2 is required for glucose synthesis and ^{13}C discrimination is reduced, thus raising $\delta^{13}C$. Clouds limit solar radiation, causing a drop in $\delta^{13}C$, along with reduced C sequestration and photosynthetic assimilation (Alton, 2008). Therefore, records of $\delta^{13}C$ from *Pinus* trees growing near the Arctic Circle in Fennoscandia show strong correlations with cloudiness, allowing $\delta^{13}C$ from tree-ring cellulose to be used as a proxy for cloud cover (Young et al., 2010, 2012; Johnstone et al., 2013).

A common problem with studies of $\delta^{13}C$ in modern tree rings is related to the Suess effect, which describes the modern day $\delta^{13}C$ decline due to the addition of fossil fuel CO_2 to the

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atmosphere (McCarroll and Loader, 2004). Because fossil fuels are derived from plant matter, which discriminates against ^{13}C, the global average carbon isotope ratio ($\delta^{13}C_{atm}$) has dropped from a pre-industrial average of –6.4 ‰ to the modern average around –8 ‰ (McCarroll and Loader, 2004; McCarroll et al., 2009). In the early Eocene (*ca.* 53.3 Ma), $\delta^{13}C_{atm}$ was –5.7 ‰ based on isotopes of benthic foraminifera sampled from North Atlantic ocean sediments in locations where surface waters sink to the ocean floor and are well mixed by the thermohaline circulation (Tipple et al., 2010). Thus, $\delta^{13}C$ estimates from these benthic foraminifera record an archive of surface water productivity levels, which are influenced by $\delta^{13}C_{atm}$ (Zachos et al., 2001). Whereas $\delta^{13}C_{atm}$ varied on millennial timescales throughout the Cenozoic, it probably did not vary significantly throughout the life of the trees in this study.

Analysis of $\delta^{18}O$ and $\delta^{13}C$ measured simultaneously from tree-ring cellulose ("dual-isotope" analysis) may help constrain paleoclimatic signals better than a single isotopic ratio alone. As some environmental factors influence both $\delta^{18}O$ and $\delta^{13}C$ through stomatal controls, and other factors affect the isotopes independently, analyzing both isotopes together offers the possibility of teasing apart environmental factors. Conceptual models of dual-isotope behavior in tree rings in response to a range of environmental factors have been proposed (Scheidegger et al., 2000) and tested (Roden and Farquhar, 2012), with theorized relationships holding true in some cases. For example, factors affecting stomatal control influenced both $\delta^{18}O$ and $\delta^{13}C$. Changing RH and keeping all other variables fixed showed that $\delta^{18}O$ and $\delta^{13}C$ are indeed positively influenced by RH, leading to the positive correlation between $\delta^{18}O$ and $\delta^{13}C$ observed in trees growing in arid regions (Saurer et al., 1995, 1997). Low RH causes $\delta^{18}O$ to increase through evaporative loss of ^{16}O molecules (H_2O molecules are smaller than CO_2 molecules, hence stomata have a reduced effect compared to CO_2) (McCarroll and Loader, 2004). In water-stressed trees, leaf stomata have a strong control over the signals of both isotopes (Saurer et al., 1995); therefore dual-isotope series show a positive correlation with each other through time (Saurer et al., 1997; Liu et al., 2014). However, trees that grow in moist regions are typically not water-stressed, so other factors not related to stomata are more likely to be dominant. For instance, low light treatments affected $\delta^{13}C$ significantly, but not $\delta^{18}O$, indicating that $\delta^{13}C$ may be used as a proxy for past light levels (Roden and Farquhar, 2012). In practice, records of cloud cover in Fennoscandia match very closely to tree ring $\delta^{13}C$, leading to its use as a cloud cover proxy (Young et al., 2010, 2012).

In this study, we measured tree-ring width and stable isotopes ($\delta^{18}O$ and $\delta^{13}C$) at annual and subannual resolution from tree-ring cellulose extracted from multiple samples of *Piceoxylon* mummified wood. Our goal was to investigate seasonal, inter-annual, and possibly multidecadal variability in tree growth and physiological functioning in this unique ancient ecosystem. The extinct Polar Forest system is important to study, because it may allow improvements in vegetation boundary conditions in paleoclimate and future climate models, which are currently major sources of uncertainty (Huber and Caballero, 2011). For example, prodigious forest growth in the Subarctic and Arctic may have had profound implications in positive warming feedbacks, through changes in albedo and hydrologic regimes relative to today. Low albedo would have caused direct warming, while greater transpiration by trees would have increased water vapor in the Arctic atmosphere, which is a powerful greenhouse gas (Beerling and Franks, 2010; Jasechko et al., 2013). Therefore, Arctic temperature amplifications during equable climates may be partially explained by transpiration-related increases in water vapor.

3. Methods

3.1 Sample materials and cellulose extraction

Paleolatitude of the Ekati Panda kimberlite 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. Samples of *Piceoxylon* wood 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 trend from the raw ring width series and strengthen the underlying climate signal. While 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 (see Supporting Information for plot data).

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We dissected individual tree rings into subannual samples (ranging from n = 5 to n = 11) 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, time series 86 y long). Kimberlite minerals were removed from the outer bark edge of samples and cross-sections (3 cm thick) were cut.

Then transects were cut from the cross-sections from pith to bark, perpendicular to tree-ring boundaries. Transects were mechanically cleaned of kimberlite minerals, and then dissected into annual or sub-annual samples using a reflected-light microscope. Individual samples were placed in sterile glass vials and ground with a micro-pestle.

We used a Modified Brendel cellulose extraction method, a heated acid hydrolysis (*via* strong nitric/acetic acids) at 120 °C for 1 hour to ensure complete delignification. Following that, we used a 2.5 % NaOH to remove hemicelluloses, which have exchangeable oxygen atoms that may be replaced by ambient (modern) oxygen and bias the signal (Brendel et al., 2000; Gaudinski et al., 2005; Richter et al., 2008a; Hook et al., 2015). Stable isotope ratios were measured at the Stable Isotope Laboratory at the University of Maryland. Cellulose was converted to carbon monoxide CO at 1080 °C over glassy carbon within a stream of 99.99 % He. Sample gas was then passed through traps for CO_2 and H_2O, and CO separated from N_2 by gas chromatography, before isotopic analysis on Continuous-Flow Micromass/Elementar Isoprime coupled to a Costech Analytical High Temperature Generator and Elemental Combustion System (Werner et al., 1996). Carbon and oxygen isotopic data were corrected for runtime drift, amplitude dependence and scaling using widely separated working cellulose isotopic standards calibrated to international reference materials (Vienna Pee Dee Belemnite, VPDB for $\delta^{13}C$, and Standard Mean Ocean Water, SMOW, for $\delta^{18}O$). The overall precisions for the corrected data, based on replicate standard analyses, are 0.14 ‰ for $\delta^{13}C$ and 0.23 ‰ for $\delta^{18}O$.

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3.2 Oxygen isotope analysis

To estimate early Eocene temperatures, the stable isotopic composition of $\delta^{18}O$ in tree ring cellulose ($\delta^{18}O_{cellulose}$) was used to estimate $\delta^{18}O$ of source water ($\delta^{18}O_{sw}$) using mechanistic models developed with modern plants (Roden et al., 2000). The Roden cellulose model uses a leaf-water $\delta^{18}O_{leaf}$ model to predict from $\delta^{18}O$ of source water (Flanagan et al., 1991) using Eq. 1:

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

where R_{wx} and R_{wa} are the molar ratios of $^{18}O/^{16}O$ in leaf water, xylem water, and atmospheric water, respectively, α is the fractionation factor for liquid-vapor equilibrium of water, which depends on temperature (Majoube, 1971), α_k is the kinetic fractionation of water ($^{16}O/^{18}O$ = 1.0285), and e_i and e_a are the partial pressures of water vapor in leaf intercellular spaces and in the atmosphere, respectively. Through a sensitivity analysis we found that the model was insensitive to changes in temperature, so we used optimal leaf temperature during photosynthesis (21.4 °C, Helliker and Richter, 2008) for calculation of α. Relative humidity (RH), however, had a large influence on the outcome, so we used a range of likely RH values in a temperate rainforest (64, 77, 83 %). The Roden et al. (2000) model uses the Flanagan et al. (1991) leaf-water model to predict $\delta^{18}O_{cellulose}$ following Eq. 2:

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

Here f_O is the fraction of carbon-bound oxygen that is subject to isotopic exchange (42 %), $\delta^{18}O_{wx}$ is the isotope ratio of xylem water and ε_O is the biochemical fractionation factor related to conversion of sugar into cellulose (27 ‰). Xylem water is used as a close approximation to source water, which is valid because no fractionation occurs between soil water and the transference to xylem water (Barbour et al., 2002). Anderson et al., (2002) created a simplified model that combined the Flanagan et al. (1991) leaf-water model with the Roden et al. (2000) cellulose model, and reversed it to solve for $\delta^{18}O_{sw}$ using $\delta^{18}O_{cellulose}$ following Eq. 3:

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

Here f is a dampening factor related to isotopic fractionations between photosynthate and stem water and h is relative humidity. In addition to these mechanistic models, we used several transfer functions developed using modern tree-ring $\delta^{18}O_{cellulose}$ and its relationship to $\delta^{18}O_{sw}$ (Ballantyne et al., 2006, Richter et al., 2008b, Csank et al., 2013). A temperature–$\delta^{18}O_{sw}$ relationship developed for the Eocene was used to estimate the MAT based on $\delta^{18}O_{sw}$ (Fricke and Wing, 2004) (Table 1).

3.3 Carbon isotope analysis

Isotopic discrimination against ^{13}C during photosynthesis has been modeled by Farquhar et al. (1982, 1989) following Eq. 4:

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

where Δ is the discrimination against ^{13}C, a is the fractionation due to diffusion through air (4.4 ‰), b is the fractionation due to carboxylation by RuBisCO (27 - 30 ‰), c_i and c_a are the partial pressures of CO_2 in the leaf intercellular spaces and atmosphere, respectively. Additionally, Δ can be calculated by Eq. 5 (Farquhar et al., 1989):

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

where $\delta^{13}C_{atm}$ and $\delta^{13}C_p$ are the carbon isotope ratios of atmospheric CO_2 and bulk plant tissue, respectively. To estimate $\delta^{13}C_{atm}$ from $\delta^{13}C_{cellulose}$ one may follow Eq. 6:

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

where ε_{pc} is the carbon isotopic difference (‰) between cellulose ($\delta^{13}C_{cellulose}$) and bulk plant 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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‰ higher (more enriched) than $\delta^{13}C$ of bulk plant tissue in the modern pCO_2 environment (Barbour et al., 2002). Early Eocene-aged mummified *Piceoxylon* ε_{pc} values fell within the modern ε_{pc} range, and are used in our calculations (ε_{pc} = 3 ‰; Hook et al., 2015). The parameters *a* and *b* in the Farquhar et al., (1982) model (Eq. 4) are usually assumed to be constant, making Δ dependent on the ratio of pCO_2 inside *v.* outside the leaf (c_i/c_a), which is unknown for the Eocene. However, Δ could be estimated using $\delta^{13}C_{atm}$ from Eq. 6, then c_i/c_a by Eq. 4. The relationship between carbon isotope ratios of plant matter ($\delta^{13}C_p$) and the atmosphere ($\delta^{13}C_{atm}$) derived by Arens et al., (2000), following Eq. 7:

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

Lomax et al. (2012) estimated the $\delta^{13}C_{atm}$–$\delta^{13}C_{cellulose}$ relationship using growth chamber experiments, given by Eq. 8:

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

As these equations are both based on empirical datasets that do not cover the full range of early Eocene pCO_2, they may not represent the "true" relationship between $\delta^{13}C_{atm}$ and $\delta^{13}C_p$ at all c_a levels. Therefore, we analyze them both as a possible range of values, and also take the arithmetic mean of Eq.'s 7 and 8, which is given by Eq. 9:

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

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 solved for Δ, then solved for c_i/c_a by rearranging Eq. 4, using Δ estimates and standard fractionation constants (*a* = 4.4, *b* = 27; Farquhar et al., 1989). We then calculated intrinsic water use efficiency (iWUE), a measure of carbon gained *vs.* water lost through stomatal

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apertures (Farquhar et al., 1982; 1989; Gagen et al., 2011) from Eq. 10, using c_a = 915 ppmV (Schubert and Jahren, 2013).

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

3.4 Dual-isotope analysis

Oxygen isotopes in cellulose are typically enriched by 20 to 30 ‰, whereas carbon isotopes are depleted (–20 to –25 ‰ range). Therefore, to make the isotopes more comparable, both datasets were normalized (mean = 0, variance = 1) and plotted together on one axis. The normalized isotope time series were then summed (subtracted) to amplify (suppress) isotopic variability common to both isotopes, and suppress (amplify) factors to which the isotopes do not respond in a similar manner. For instance, changes in stomatal conductance (*e.g.,* due to changes in relative humidity or drought) affect both isotopes, so the dual-isotope time series should be positively correlated and vary in-phase with each other (Saurer et al., 1997). Any variance in the dual-isotope series that is not explained by this positive correlation is likely related to other factors. A factor that would likely influence $\delta^{13}C_{cellulose}$ (but not $\delta^{18}O_{cellulose}$) is a reduction in light, possibly by cloud coverage (Johnstone et al., 2013). On the other hand, $\delta^{18}O_{sw}$ would significantly affect $\delta^{18}O_{cellulose}$ (but not $\delta^{13}C_{cellulose}$) (Ferrio and Voltas, 2005).

One way to amplify an environmental signal common to two proxies is addition. Adding the normalized series together ($\Sigma_{Z\text{-score}}$) amplifies the in-phase components of the variance, and suppresses the out-of-phase components. Conversely, subtracting the dual-isotope series from each other ($\Delta_{Z\text{-score}}$) amplifies the out-of-phase components of the variance and suppresses the in-phase components. Principal Components Analysis (PCA) was conducted on the dual-isotope dataset to examine the variance structure. PCA on two variables produces a two-dimensional

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plot of two eigenvectors: PC1 and PC2, which are orthogonal to each other and identify factors that explain the most variance between the isotopes (PC1), as well as variance that is uncorrelated between the two datasets. Therefore, PC1 corresponds with $\Sigma_{Z\text{-score}}$, and PC2 with $\Delta_{Z\text{-score}}$, as described above. Spectral analysis was conducted [Multi-Taper Method, MTM (Mann and Lees, 1996); Singular Spectral Analysis, SSA (Vautard and Ghil, 1989); kSpectra software] on the raw data, PC1 ($\Sigma_{Z\text{-score}}$), and PC2 ($\Delta_{Z\text{-score}}$) time series to examine the temporal power spectra.

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4. Results and discussion

Tree ring growth was prodigious in the earliest Eocene Subarctic [mean tree ring width for the *Piceoxylon* samples ranged from 1.88—2.19 mm (σ range = 0.65—0.76)]. However, ring width series in this study were sensitive enough for crossdating (mean sensitivity values = 0.20—0.36). The overlapping ring sequences from the wood fragments were positively correlated, supporting the idea that the trees were subjected to similar climatic conditions (EPA3 *v.* EPA4, R = 0.38, p = 0.04, n = 30). Some ring width series were so similar that they may have originated from the same tree (EPA4 *v.* EPA6, R = 0.90, p < 0.0001, n = 35). Annual-resolution dual-isotope series were strongly correlated in both overlapping sections with regard to $\delta^{18}O$ (EPA3 *v.* EPA4, R = 0.78, p < 0.0001, n = 22; EPA4 *v.* EPA6, R = 0.85, p < 0.0001, n = 31) (lower two graphs in Figure 1). One of the overlapping sections of $\delta^{13}C$ was strongly correlated (EPA3 *v.* EPA4, R = 0.73, p < 0.0001, n = 22), but the other was strongly non-correlated (EPA4 *v.* EPA6, R = 0.01, p = 0.97, n = 31). Both the RWI and $\delta^{18}O$ records correlate strongly in this section so it is unknown why $\delta^{13}C$ does not. Cross-correlation analysis of RWI and isotope series suggests that climatic conditions from the previous year or two significantly influence tree-ring width [$\delta^{18}O$ lagged –1 year before RWI (R = 0.27, p = 0.02, n = 84), $\delta^{18}O$ lagged –2 years before RWI (R = 0.22, p = 0.04, n = 83)]. Additionally, a positive correlation was found when $\delta^{18}C$ was lagged +2 with regard to RWI (R =

0.23, p = 0.04, n = 83). This correlation may indicate that increased tree-ring growth is associated with increased foliage production in the following years, thus leading to an increase in photosynthetic capacity and hence an increase in $\delta^{13}C$.

Days were long in the subarctic summer (~19 hr/d at summer solstace), allowing high rates of photosynthesis, provided solar radiation was not obscured by clouds. In the subannual study, the intra-annual series generally showed a rise and fall pattern throughout the growing season, suggesting that this wood is of a persistent-leaved species (upper two graphs in Figure 1) (Barbour et al., 2001). Earlywood cellulose in deciduous species is isotopically enriched in $\delta^{13}C$ compared to persistent-leaves species, due to the use of carbohydrates stored in parenchyma over the dormant season (Jahren and Sternberg, 2008). Changes in relative humidity (RH) may be explained by a positive slope in a scatterplot of $\delta^{18}O$ and $\delta^{13}C$ (Roden and Farquhar, 2012). Theoretically, lowest RH (highest T) would be in midsummer when the continuous light regime is near its peak (Figure 2). However, other factors besides RH probably affected the isotope signals in most years not described by a simple rise and fall pattern along the RH slope. Tree ring (TR) 39 displayed a small range in $\delta^{18}O$ (1.7 ‰) and $\delta^{13}C$ (0.4 ‰) throughout the year possibly indicating mild homogenous climate during that year (Figure 2). On the other hand, years with high solar radiation but lower temperature variation may have raised the $\delta^{13}C$ without significantly altering $\delta^{18}O$, as in the end of the season in TR 40. The range in $\delta^{18}O$ in ring 42 (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 42 include an amount effect due to progressively heavier 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 42, which may have benefitted

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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 40 and 42, 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).

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The annual-resolution dual-isotope record was positively correlated (Pearson's R = 0.36, P < 0.001, n = 86) (Figures 1 and 3). This suggests that stomatal conductance was an important factor in the physiological functioning of these trees (Saurer et al., 1995). However, the first 4—8 tree rings were noticeably lower in $\delta^{13}C$ 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). Using the $\delta^{18}O$ 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 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 (~16.4 ± 3.0 °C), which is in agreement with published records of high Arctic seasonal temperatures (19—20 °C, Eberle et al., 2010). Because tree-ring growth ceases during the winter, cold month mean temperatures (CMMT) cannot be directly calculated with this proxy. However, if independent estimates of CMMT based on Eocene MAT could be applied to our study. Estimates based on apatite of bowfin (*amiid*) fish that grow year-round suggest CMMT of 0—3.5 °C and an MAT of 8 °C (Eberle et al., 2010). In our annual study, the mean of all of the methods (black line in Figure 5) ranged from 7.5—16.6 °C, with a mean of 11.4 °C (1 σ = 1.8 °C) (Table 2). This would suggest a CMMT of ~3.4—6.9 °C during the earliest Eocene based on the findings of Eberle et al., (2010) applied to our MAT estimate. The standard deviation of all methods was 4.1 °C, and the 90 % confidence interval was 2.7 °C (Figure 5). A mean temperature of 11.4 °C is close to other estimates of early Eocene MAT based on independent proxies (*e.g.,* leaf margin analysis: 11—14 °C, Sunderlin et al., 2011). Some of the highest MAT estimates produced (> 20 °C) match estimates of warmest mean temperatures for the early Eocene (18—20 °C) (Weijers et al., 2007). Our MAT estimate is 2.4 °C higher than that of Wolfe et al. (2012) (grand mean = 9 °C), but our mean estimate of 11.4 °C falls within the total range of MAT estimates provided by that study (7—12 °C). Their study was conducted on cellulose from *Metasequoia* trees from the same kimberlite mine (n = 4). However, bulk wood samples were taken in that study, precluding the possibility of examining climates from distinct years. We measured 141 individual tree rings from three crossdated tree-ring series spanning an

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86-year-long period, and there were years in our record in which the MAT estimate was as low as 9 °C as in Wolfe et al. (2012). It may be that the cellulose sampled in that study grew during these years of slightly lower MAT, or that differences of 1—3 °C are not currently resolvable using these proxies and the values are essentially equivalent.

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.

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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) increases strongly; Scenario (2) c_i increases proportionally to c_a, causing c_i/c_a to remain relatively constant and iWUE to increase; Scenario (3) c_i increases at about the same rate as c_a, and c_i/c_a increases while iWUE remains constant. In free air carbon enrichment (FACE) plots, c_i/c_a tends to decrease slightly (–0.02 to –0.08 %), but significantly, in high pCO_2 (~600 ppmV) with respect to control plots (~400 ppmV), supporting Scenario (1) above (Battipaglia et al., 2013). However, the opposite pattern is found in controlled growth chamber experiments (Lomax et al., 2012; Schubert and Jahren, 2012). Using strict controls over hydrologic variables (*i.e.,* relative humidity, soil water potential), Schubert and Jahren (2012) found that Δ is positively related to pCO_2 by a hyperbolic function, such that Δ does not increase infinitely with increasing pCO_2 as with a linear function, but flattens out as it approaches a limit of 28.26 ‰. This increase in Δ may increase active carboxylation sites on RuBisCo, thus increasing c_i/c_a, which would support Scenario (3) (Schubert and Jahren, 2012). However, these growth-chamber experiments were designed to identify the relationship between Δ and pCO_2 at a constant stomatal density (SD = number of stomata per unit area on the leaf). During the Eocene SD was lower than modern SD in response to higher pCO_2, which would have affected gas exchange and water use efficiency (Beerling et al., 2009).

Stomatal density or stomatal index (SI) of fossil leaves have long been used as paleo-pCO_2 proxies based on the observation that plants decrease SD and SI in high pCO_2 (Beerling et al., 1998) and vice versa (Woodward, 1986; 1987) following a negative hyperbolic relationship that flattens out at high pCO_2 levels (Royer, 2003; Beerling et al., 2009), mirroring the hyperbolic relationship between Δ and pCO_2 (Schubert and Jahren, 2012). SD and SI display remarkable phenotypic and genotypic plasticity to changing atmospheric pCO_2 over both short-term (*i.e.,* hours to months) and long-term (*i.e.,* evolutionary) timescales (Beerling and Chaloner, 1993). Reducing SD/SI during high pCO_2 maximizes efficiency in CO_2 uptake by leaf stomata, while minimizing water loss, thus resulting in iWUE over twice as much as modern iWUE in high-latitude *Pinus* trees < 100 μmol mol^{-1}; Gagen et al. 2011). Greenhouse experiments with *Pinus sylvestris* L. trees at elevated pCO_2 (560 ppmV) and temperature (+3 to 5 °C) show no change in c_i/c_a despite reduced SD and increased iWUE (Beerling, 1997). Moreover, manipulations of SD *via* epidermal patterning factor (EPF) genes in *Arabidopsis* mutants suggest that reduced (increased) SD may lead to decreased (increased) transpiration and stomatal conductance (g_s), along with increased (decreased) growth and iWUE (Doheny-Adams et al., 2012). Lower SD causes reductions in c_i/c_a, which increases iWUE without changing photosynthetic capacity (Franks et al., 2015). This optimizes operational stomatal conductance (g_{sop}) around a "sweet spot" of 20 % maximum anatomical conductance (g_{smax}) (Dow et al., 2014). By operating at around 20 % of g_{smax}, stomatal guard cells can be more responsive to rapid environmental changes in RH or VPD. Therefore, the opposing hyperbolic curves (Δ vs. pCO_2, SD vs. pCO_2) may balance out as a result of this phenotypic and genotypic plasticity, stabilizing Δ and c_i/c_a through geologic time (Ehleringer and Cerling, 1995; Dawson et al., 2002), supporting Scenario (2) above (Saurer et al., 2004).

In the modern climate, the Suess effect greatly alters $\delta^{13}C_{atm}$, curving it unnaturally downward starting with the industrial revolution, so tree ring records spanning this period must be isotopically corrected (McCarroll et al., 2009). However, in the early Eocene average $\delta^{13}C_{atm}$ levels were likely to be constant over the life of a tree in the absence of a hyperthermal event (Zachos et al., 2001). Therefore, any shifts upward or downward around the mean $\delta^{13}C_{cellulose}$ are probably related to annual or seasonal changes in photosynthetic rate (*A*) or stomatal

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conductance (g_s), both of which influence c_i/c_a. Photosynthesis would not have affected by high pCO_2 under the continuous light of the polar summer (Beerling and Osborne, 2002), but may have been affected by cloud-related reductions in sunlight (Young et a. 2010). We assume our $\delta^{13}C_{cellulose}$ record to be a qualitative proxy of sunlight/cloudiness, with the exception of a brief period during the juvenile phase when trees must compete for light in the shaded understory, leading to a juvenile effect in the early part of some $\delta^{13}C$ records (Gagen et al., 2007).

Although precise quantitative estimates of sunlight cannot be made, analysis of both isotopes simultaneously can aid in qualitative assessment of solar variability. When both isotope datasets are normalized (Figure 6, top graph) and summed (Figure 6, middle graph), a signal related to RH and vapor pressure deficit (VPD) should be amplified, because both isotopes are affected by g_s [low RH (high VPD) causes an increase in both $\delta^{18}O_{cellulose}$ and $\delta^{13}C_{cellulose}$, leading to a positive correlation (Saurer et al., 1995)]. Conversely, when the dual isotope data are normalized and subtracted, the remaining unexplained variance relating to factors other than RH should be amplified (Figure 6, bottom graph). For $\delta^{18}O_{cellulose}$, $\delta^{18}O_{sw}$ is a major factor (related to temperature of precipitation and precipitation sources), and for $\delta^{13}C_{cellulose}$ cloudiness is the most likely controlling factor because clouds limit photosynthetic rate. Modern trees growing near the Arctic Circle in Fennoscandia show high correlations between annual records of stable carbon isotope ratios ($\delta^{13}C$) and records of cloud cover, where the dominant factor in their $\delta^{13}C$ records is photosynthetic rate (Young et al., 2010, 2012). When more sunlight is received, photosynthetic rate is increased, which reduces isotopic discrimination and raises the $\delta^{13}C$ value. However, a converse relationship exists between sunlight and temperature at different timescales. Proxy records suggest that at high frequency (annual) timescales, sunlight and temperature are positively related (*i.e.,* sunny = warm, cloudy = cool), but at low frequencies (multidecadal), they are negatively related (*i.e.,* cloudy = warm, sunny = cool) (Young et al.,

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2012). This is somewhat counterintuitive but sustained, regional warmer temperatures cause an increase in evaporation and cloud cover, bringing latent heat to northern latitudes through increased precipitation. Simultaneously, clouds cause short-term local cooling by blocking solar radiation.

Spectral analysis of the normalized summed data (PC1) shows a significant interannual-scale pattern (2—3 ypc) (Figure 6, middle graph), whereas the normalized subtracted data (PC2) shows multidecadal cyclicity (20—30 ypc) (Figure 6, bottom graph). This pattern is similar to modes of the modern Pacific Decadal Oscillation (PDO) and Arctic Oscillation/North Atlantic Oscillation (AO/NAO), which operate on multidecadal time-scales (Mantua et al., 1997, Young et al., 2012). These modes are also teleconnected with ENSO cycles (2—7 ypc) in the modern climate (Gershunov and Barnett, 1998). Temperature increases during positive phases of the PDO contribute to greater evaporation, leading to enhanced cloud formation and precipitation levels on a strongly bidecadal mode (Chiacchio et al., 2010). Sparse cloud cover may not significantly block sunlight, as diffusion may redistribute it through the canopy (Reinhardt et al., 2010; Urban et al., 2012). However, if cloud cover is very dense it may limit tree growth by blocking photons necessary for photosynthesis (Ritchie, 2010). Heavy cloud cover has been implicated in reduced photosynthetic rate of modern black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenburg) growing at subarctic treeline in Quebec, Canada (Vowinckel et al., 1975).

When dual-isotope analyses [PC1 ($\Sigma_{\text{Z-score}}$), and PC2 ($\Delta_{\text{Z-score}}$)] were compared with RWI data, an apparent positive association existed between PC2 and RWI at low frequencies. The middle portion (*i.e.,* tree rings least likely affected by juvenile growth or diagenetic factors) of the 7-year running mean data was strongly positively correlated (TR 27—82; $R = 0.68$, $p < 0.0001$, $n = 55$) (Figure 7). This suggests that PDO-like climate fluctuations of temperature and precipitation led to decades of increased tree growth during positive phases of the early Eocene “PDO”, and decades of decreased growth during negative “PDO” phases. No association was found between PC1 and RWI records. If PC1 (positive correlation of isotopes) is related to Eocene RH, sustained high humidity may explain this non-association (*i.e.,* low RH variability, Saurer et al., 1995).

In the early Eocene, subarctic trees may have been strongly dependent on both light and precipitation, and therefore influenced by cloud coverage. Sewall and Sloan (2001) hypothesized that in the Eocene, the lack of polar ice contributed to a stable positive Arctic Oscillation, rather than the multidecadal dipole that currently exists. However, the RWI and isotope data presented here suggest that PDO-like cyclicity operated in the early Eocene, possibly contributing to AO teleconnections as it does today (Jia et al., 2009). Oceanic Rossby waves may have set the timescale for multidecadal shifts in the position of the Aleutian low-pressure system, which changes the trajectory of weather patterns (Gershunov and Barnett, 1998). During positive PDO phases the position of the Aleutian low shifts southward, drawing in ENSO-mediated tropical moisture and delivering it to the Subarctic (Figure 8). Another possibility for the $\delta^{18}O$ variation is multidecadal shifts in source water location (*e.g.,* Pacific Ocean, Arctic Ocean). In the early Eocene the Arctic Ocean was isolated from other oceans, with high freshwater content from high precipitation (Brinkhuis et al., 2006). Thus, the Arctic Ocean source water would have been depleted in $\delta^{18}O$ relative to Pacific Ocean source water. Therefore, the trees in our study may have alternately received low-$\delta^{18}O$ from the Arctic, and high-$\delta^{18}O$ from the Pacific shifting every 20—30 years.

Jahren and Sternberg (2002) suggested that meridional transport of precipitation northward across the North American continent could have depleted the $\delta^{18}O$ of rainwater before reaching their study site. However, such a strong southerly wind current system seems unlikely in the Eocene, if the latitudinal temperature gradient was low (Greenwood and Wing, 1995), and given similar orbital variability (Laskar et al., 2011). However, if Eocene equatorial temperatures were high (35—40 °C, Caballero and Huber, 2010) temperature gradients may have been stronger than previously thought, leading to strong winds. Another possible explanation for the low $\delta^{18}O$ values of extreme northern polar forests in that study is that the source water was largely recycled from depleted Arctic Ocean sources, or water transpired from trees (Jasechko et al., 2013). Additionally, mineral contamination (*e.g.,* by iron oxides) may also cause negative $\delta^{18}O$ errors (Richter et al., 2008a). Paleoclimate models suggest that increases in atmospheric water vapor due to an ice-free Arctic may have created conditions conducive to formation of a stable Arctic cyclone, through which southern precipitation sources could not penetrate (Sewall and Sloan, 2001). Our results suggest that if this stable Arctic cyclone existed then it probably still had teleconnections with a PDO-like mechanism, causing the edge of the cyclone to shift northward and southward on multidecadal timescales.

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5. Conclusions

Multiple tree-ring based proxies were examined to study the climate of the early Eocene. The material used was extremely well preserved *Piceoxylon* Gothan 1905 mummified wood found in kimberlite diamond mines (*ca.* 53.3 Ma), which allowed geochemical investigations of primordial cellulose. Stable isotope data ($\delta^{18}O$ and $\delta^{13}C$) were collected from subannually and annually sampled increments along tree-ring chronologies. Mean annual temperatures (MAT) were estimated to be 11.4 °C using $\delta^{18}O$ isotopes, taking the mean of a variety of commonly used mechanistic models (Roden et al., 2000; Anderson et al., 2002) and transfer functions (Ballantyne et al., 2006; Richter et al., 2008b; Csank et al., 2013) designed for estimating temperature with wood cellulose. This value is in agreement with other studies using alternate proxies (Greenwood and Wing, 1995; Sunderlin et al., 2011). The range is 7.5—16.6 °C, which is 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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 ($\Sigma_{Z\text{-score}}$). 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. 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 $\delta^{18}O$ of source water, and $\delta^{13}C$ shifts related to cloudiness regimes on bidecadal (20—30 ypc)

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timescales. The fact that these timescales are similar to a modern day PDO frequency spectrum suggests modern climate dynamics are similar to those experienced during the earliest Eocene, despite pronounced global warmth.

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Author Contributions

B.A.H. designed study, collected and analyzed data, wrote manuscript, J.H. edited manuscript, Z.G. edited manuscript, J.B. edited manuscript, D.J.S. edited manuscript.

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Group, when Panda Pipe samples were donated, Ekati Diamond Mine was owned by BHP Billiton), for access to sample materials; U. Fekl and U. Wortmann for access to equipment; R.E. Plummer, K.J. Steele and M.N. Evans at the University of Maryland Stable Isotope laboratory for conducting mass spectrometry. J. Basinger for helpful editing suggestions.

Figure 1. Subannual and annual-resolution time series records of tree-ring cellulose $d^{18}O$ and $d^{13}C$. Subannual resolution a) $\delta^{18}O$ record, and b) $\delta^{13}C$ record, of four tree rings (TR 39—42). Lines above and below the measured values (bold center lines) show the analytical uncertainty (0.14 ‰ for $\delta^{13}C$, 0.23 ‰ for $\delta^{18}O$). Annual resolution c) $\delta^{18}O$ record, and d) $\delta^{13}C$ record (n = 85). 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 21—54, 57—75).

Figure 2. Scatterplots of dual-isotope data for four tree rings (TR 39—42), showing trends of $\delta^{18}O$ and $\delta^{13}C$ 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: [$\delta^{13}C = 0.22 * \delta^{18}O - 31.31$]. Scale is the same for inset graph, but actual values of Roden and Farquhar, 2012 ($\delta^{18}O$ low RH = 29.26 ‰, $\delta^{18}O$ high RH = 26.9 ‰; $\delta^{13}C$ low RH = –24.86 ‰, $\delta^{18}O$ high RH = –25.38 ‰) do not correspond with these axes.

Figure 3. Correlation analysis of dual-isotope annual dataset. $\delta^{18}O$ and $\delta^{13}C$ 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 $\delta^{18}O$-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 $\delta^{18}O$-temperature reconstructions. Mean of all reconstructions (black line) is bracketed by 90 % confidence interval (± 90 % ci, dark gray fill), one

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

Figure 6. Results of dual-isotope ($\delta^{18}O$ and $\delta^{13}C$) analysis (ISO chronology, n = 86). *Upper panel:* Normalized $\delta^{18}O$ ($\delta^{18}O_{Z\text{-score}}$, thin gray line) and $\delta^{13}C$ ($\delta^{13}C_{Z\text{-score}}$ thin black line), and 7-yr triangular running mean $\delta^{18}O_{Z\text{-score}}$ (bold gray line) and $\delta^{13}C_{Z\text{-score}}$ (bold black line). *Center panel:* Sum of $\delta^{18}O_{Z\text{-score}}$ and $\delta^{13}C_{Z\text{-score}} = \Sigma_{Z\text{-score}}$ (thin gray line), and 7-yr triangular running mean (bold gray line). *Lower panel:* Difference of $\delta^{18}O_{Z\text{-score}}$ minus $\delta^{13}C_{Z\text{-score}} = \Delta_{Z\text{-score}}$ (thin black line), and 7-yr triangular running mean (bold black line). Shaded regions in upper and lower panels highlight the bidecadal oscillations especially evident in the PC2 ($\Delta_{Z\text{-score}}$) chronology in the lower panel.

Figure 7. Correspondence of *Piceoxylon* tree-ring width indices (RWI) and stable isotope chronologies. (*Upper*) *Piceoxylon* RWI (n = 92, gray line) with 7-year triangular running mean (bold black line) to highlight low-frequency variability. (*Lower*) *Piceoxylon* isotope PC2 chronology (n = 86, gray line) with 7-year triangular running mean (bold black line) to highlight low-frequency variability. Here, grey boxes denote warmer and cloudier decades with above average tree ring growth. The first seven tree rings of the RWI record were not analyzed for stable isotopes, due to concerns about possible influences of juvenile tree growth on the isotope record. Question mark at the beginning of the TR record depicts uncertainty due to a possible juvenile growth signal.

Figure 8. Position and strength of Aleutian low-pressure system during positive and negative phases of the PDO in relation to study site. Hypothesized stable Arctic Oscillation during the Eocene depicted by grey arc in upper right corner (*see Sewall and Sloan 2001). 1000 mb sea level pressure (SLP) contours shown for negative PDO (blue shaded area) and positive PDO (red shaded area). Weather patterns are altered according to these changes in SLP (blue arrow – negative PDO, red arrow – positive PDO), thus altering the distribution of precipitation across North America. Positions of 1000 mb contours of Aleutian low after NOAA-CIRES/Climate Diagnostics Center (Jan-Mar sea level pressure (mb) composite for negative PDO 1988, 1999; for positive PDO 1983, 1987, 1992, 1998).

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

$$T\,(°C) = (0.000005 * \delta^{18}O_{sw}{}^4) + (0.0007 * \delta^{18}O_{sw}{}^3) + (0.0436 * \delta^{18}O_{sw}{}^2) + (2.1153 * \delta^{18}O_{sw}) + 32.697$$

Table 2. Early Eocene Mean Annual Temperature (MAT) estimates based on $\delta^{18}O$ of *Piceoxylon* cellulose. Several methods of temperature estimation in the literature were used, including mechanistic models (Roden et al., 2000; Anderson et al., 2002) and transfer functions (Csank et al., 2013; Richter et al., 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 $\delta^{18}O_{sw}$–temperature relationship developed for the Eocene (Fricke and Wing, 2004). Shown are references for model/function, relative humidity level (*for mechanistic models*), range (min—max) of MAT (°C), and 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)***

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

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