

Supplementary Information

Supplementary Methods:

Based upon a global survey of modern soils (Weijers et al., 2007b), it has been determined that the chemical structure of tetraethers, containing 4-6 methyl groups and 0 to 2 cyclopentyl moieties, are highly sensitive to both changes in pH and MAT. In particular, the degree of methylation, expressed in the index of methylated branched tetraethers (MBT) and the degree of cyclisation, expressed in the cyclisation ratio of branched tetraethers (CBT), was highly correlated with MAT ( $R^2=0.77$ ), such that:

$$\text{MAT} = (\text{MBT} - 0.122 - 0.187 \times \text{CBT}) / 0.02 \quad (1)$$

Even in settings with a cold climate and short growing season, predicted MAT from the MBT/CBT proxy is close to measured MAT values (Peterse et al., 2009), suggesting that tetraethers in the paleosols at the Beaver Pond Site are a viable independent proxy of MAT during the Pliocene. This proxy has been successfully used to reconstruct continental temperatures in the Arctic during the Paleocene/Eocene thermal maximum (Weijers et al., 2007a). A sample chromatogram used for calculating the degree of methylation and cyclisation among tetraethers is included as supplemental Figure DR4.

The second proxy used to reconstruct temperature in this study was based on the isotopic composition of cellulose (Brendel et al., 2000) and annual ring width of fossil larch. A multivariate model was constructed by sampling larch growing near sites with sufficient isotopic and meteorological data at the northern and southern limits of the Boreal Forest. Based on this

approach it was determined that the optimal multivariate model for predicting temperature was one that included the  $\delta^{18}\text{O}$  of precipitation ( $\delta^{18}\text{O}_{\text{pre}}$ ) and annual ring widths ( $RW$ ), such that:

$$\text{MAT} = 17.5 + 0.98 \times \delta^{18}\text{O}_{\text{pre}} - 2.71 \times RW. \quad (2)$$

Although the optimal model for predicting MAT is based on  $\delta^{18}\text{O}_{\text{pre}}$  and  $RW$ , Ballantyne et al. (Ballantyne et al., 2006) had only information on the oxygen isotopic composition of cellulose ( $\delta^{18}\text{O}_{\text{cel}}$ ) from fossil larch. Therefore, a database of modern values of  $\delta^{18}\text{O}_{\text{pre}}$  and  $\delta^{18}\text{O}_{\text{cel}}$  was compiled from the literature to calculate an isotopic discrimination ( $\Delta^{18}\text{O} = \delta^{18}\text{O}_{\text{cel}} - \delta^{18}\text{O}_{\text{pre}}$ ) of  $\Delta^{18}\text{O} = 43.0\text{‰}$  in fossil Arctic larch and ultimately  $\delta^{18}\text{O}_{\text{pre}} = -23.5\text{‰}$  for the Pliocene. However, recent measurements of  $\delta^{18}\text{O}_{\text{cel}}$  in mosses from the Beaver Pond Site allow us to estimate MAT directly (Eqn. 2). This is based on the fact that mosses have no stomata and that fractionation between meteoric water and the oxygen assimilated in the cellulose of mosses is effectively constant (Deniro and Epstein, 1981). Although fractionation of meteoric water may occur due to evaporation from the ephemeral ponds where mosses are found (Sauer et al., 2001), the Beaver Pond site was probably a more permanent hydrologic feature and thus the isotopic composition of mosses at the site integrates annual precipitation more than seasonal evaporation (Supplementary Figure DR1). To calculate  $\delta^{18}\text{O}_{\text{pre}}$ , we used the mean value of  $10.6 \pm 0.9 \text{‰}$  for  $\delta^{18}\text{O}_{\text{cel}}$  measured in mosses and the constant  $\Delta^{18}\text{O} = 27\text{‰}$  (Zanazzi and Mora, 2005), which is statistically indistinguishable from the original value reported by Deniro and Epstein (1981) for a wide range of aquatic organisms. Based on our isotopic analysis of the mosses, the isotopic composition of precipitation in the Pliocene High Arctic was probably on the order of  $-16.4 \text{‰}$ , thus indicating a lower isotopic enrichment than previously thought  $\Delta^{18}\text{O} = 36.0\text{‰}$ . This diminished isotopic enrichment in larch growing in the Arctic during the Pliocene suggests that relative humidity may have been greater than previously inferred (Ballantyne et al., 2006). In

fact, measurements of  $\delta^{18}\text{O}$  in precipitation and cellulose of modern larch growing in Ottawa, Canada (see Supplementary Fig. DR1) show a similar isotopic enrichment ( $\Delta^{18}\text{O} = 37.2\text{‰}$ ), suggesting that relative humidity in the Arctic during the Pliocene may have been similar to the modern mean annual relative humidity of Ottawa which is around 79%(Canada, 2009).

The third temperature proxy we used in this study was based on the assemblage of paleovegetation found at the site and the climatic ranges of their nearest living relatives (NLRs), using the coexistence approach of Mosbrugger and Utescher (1997). Because plant taxa are sedentary and often slower to respond to climate variability than animal taxa, they are particularly useful for climate reconstructions (Greenwood and Wing, 1995). From the list of palaeoflora identified from the site (Matthews Jr and Ovenden, 1990), we queried the databases of North American tree taxa (Thompson et al., 2000) and Natural Resources Canada (2009) for non-tree taxa, and the more extensive PALAEOFLOA database of Mosbrugger and Utescher (1997) for temperature ranges and MAT values based on the modern distribution of taxa. A total of 16 taxa identified at the site have climate range data, of which 10 were conspecifics with modern species, 3 were matched to modern taxa at the genus level, and the remaining 3 taxa were matched to similar modern species. For the coexistence approach, the coexistence zone is defined as the band of overlap for a climate parameter where all taxa can co-occur (Supplementary Table DR1). The lower bound for MAT was set by the warmest minimum MAT across the temperature ranges of all NLRs, which was *Thuja occidentalis* ( $-4.4^{\circ}\text{C}$ ) and the upper bound by the coldest maximum MAT across the temperature ranges of all NLRs, which was *Betula nana* ( $3.7^{\circ}\text{C}$ ). We used a variation on the approach by Mosbrugger and Utescher (1997), whereby the estimate of MAT provided is given as the mean of these two values and the standard error is expressed as the difference between the mean MAT and the upper and lower limits of the

MAT range (Supplemental Table DR1). This manner of expressing the estimate facilitates statistical comparisons of this estimate with those provided by the other proxies.

The bootstrap resampling technique presented here provides an excellent approach for constraining estimates of palaeoclimatic variables that are often derived from very few measurements. This approach also allows one to combine estimates from distributions of temperature estimates derived from independent proxies, provided that they are statistically indistinguishable. This approach may prove valuable for estimating parameters such as temperature sensitivity and atmospheric CO<sub>2</sub> which often yield probability density distributions that are not normally distributed (Hegerl et al., 2006).

Data compiled for generating latitudinal temperature curves for the Pliocene and the Eocene are presented in Supplementary Table DR2. All original references for temperature estimates from the Pliocene are reported (Supplementary Table DR2) and temperature estimates for the Eocene are from Greenwood et al. (Greenwood and Wing, 1995) and references therein. The modern curve was generated, by assembling all low elevation records (< 200 MASL) from the cited sources for meteorological stations on the west coasts, east coasts, and a N-S transect through each of the major continents [North America (Bryson, 1974), South America (Schwerdtfeger, 1976), Australia and New Zealand (Gentili, 1971), Africa (Griffiths, 1971), as well as Antarctica and the Arctic (Orvig, 1970)]. The curve is a polynomial function fitted to the data, according to Greenwood et al. (1995).

Supplementary Table DR1. Palaeotemperature estimates derived from independent proxies. Reported in the table are the raw data from the three independent temperature proxies, palaeovegetation, isotopes and ring widths, as well as tetraethers. In addition, estimates of mean annual temperature (MAT) from individual taxa and samples, as well as their mean ( $\bar{x}$ ) and standard deviation ( $\sigma$ ) are reported.

<u>Proxies</u>	<u>MAT<sub>min</sub> (°C)</u>	<u>MAT<sub>max</sub> (°C)</u>	<u>Data Source</u>
<u>Palaeovegetation<sup>a</sup></u>			
<i>Alnus incana</i>	-10.2	11.2	(NRC, 2009)
<i>Saxifraga oppositifolia</i>	-5.0	9.3	(NRC, 2009)
<i>Myrica gale</i>	-6.1	9.9	(NRC, 2009)
<i>Empetrum nigrum</i>	-6.3	9.5	(NRC, 2009)
<i>Andromeda polifolia</i>	-6.1	9.5	(NRC, 2009)
<i>Carex diandra</i>	-5.5	10.5	(NRC, 2009)
<i>Potentilla norvegica</i>	-5.1	14.0	(NRC, 2009)
<i>Menyanthes trifoliata</i>	-6.1	10.4	(NRC, 2009)
<i>Vaccinium vitis-idaea</i>	-6.3	9.5	(NRC, 2009)
<i>Betula nana</i>	-13.4	3.7	PALAEOFLOA
<i>Betula papyrifera</i>	-12.2	11.4	PALAEOFLOA
<i>Thuja occidentalis</i>	-4.4	14.2	PALAEOFLOA
<i>Salix 'cold'</i>	-16.3	5.9	USGS
<i>Picea mariana</i>	-12.4	10.7	PALAEOFLOA
<i>Pinus albicaulis</i>	-4.8	10.2	PALAEOFLOA
<i>Larix laricina</i>	-9.7	11.2	PALAEOFLOA
MAT range	-4.4	3.7	
$\bar{x}$ MAT	-0.4		
<u>Isotopes and Ring Widths<sup>b</sup></u>			
	<u>MAT (°C)</u>		
1	-0.7		
2	-1.0		
3	-1.3		
4	0.5		
5	-0.5		
6	-1.0		
7	-0.4		
8	-0.2		
9	0.0		
$\bar{x}$	-0.5		
$\sigma$	0.6		
<u>Tetraethers<sup>c</sup></u>			
	<u>MAT (°C)</u>		
0.1	-1.0		
0.2	-0.7		
0.3	-1.1		

0.4	-1.2
0.5	-0.6
0.6	-1.4
0.7	-0.4
0.8	1.3
0.9	-0.3
$\bar{x}$	-0.6
$\sigma$	0.6

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<sup>a</sup> Individual temperature estimates derived from the coexistence approach (Mosbrugger and Utescher, 1997) for each taxa. The mean MAT is calculated as the average of the temperature minima and maxima for the temperature range. Sources: NRC, 2009 – McKenney et al. 2007 / Natural Resources Canada ([http://planthardiness.gc.ca/ph\\_main.pl?lang=en](http://planthardiness.gc.ca/ph_main.pl?lang=en)) using ‘of interest to ecologists’ option; PALAEOFLOA (<http://www.palaeoflora.de/>) – with additional data from Torsten Utescher (*pers. comm.*); USGS – Thompson et al. 2000 online version (<http://pubs.usgs.gov/pp/p1650-b/>).

<sup>b</sup> Individual temperature estimates derived from annual measurements of oxygen isotopes and ring-widths in fossil larch trees from the site (see methods). Each MAT estimate represents one year of growth from an individual larch.

<sup>c</sup> Individual temperature estimates derived from the measurement of tetraethers in peat samples collected from sedimentary layers from the site (Supplementary Fig. DR1b). Sample descriptions correspond with elevations (m) above the base of the peat deposit at the site.

Supplementary Table DR2. Estimates of mean annual temperature (MAT) arranged by latitude. Temperature estimates from the Pliocene are from the references cited and temperature estimates from the Eocene are from Greenwood and Wing (Greenwood and Wing, 1995) and references therein.

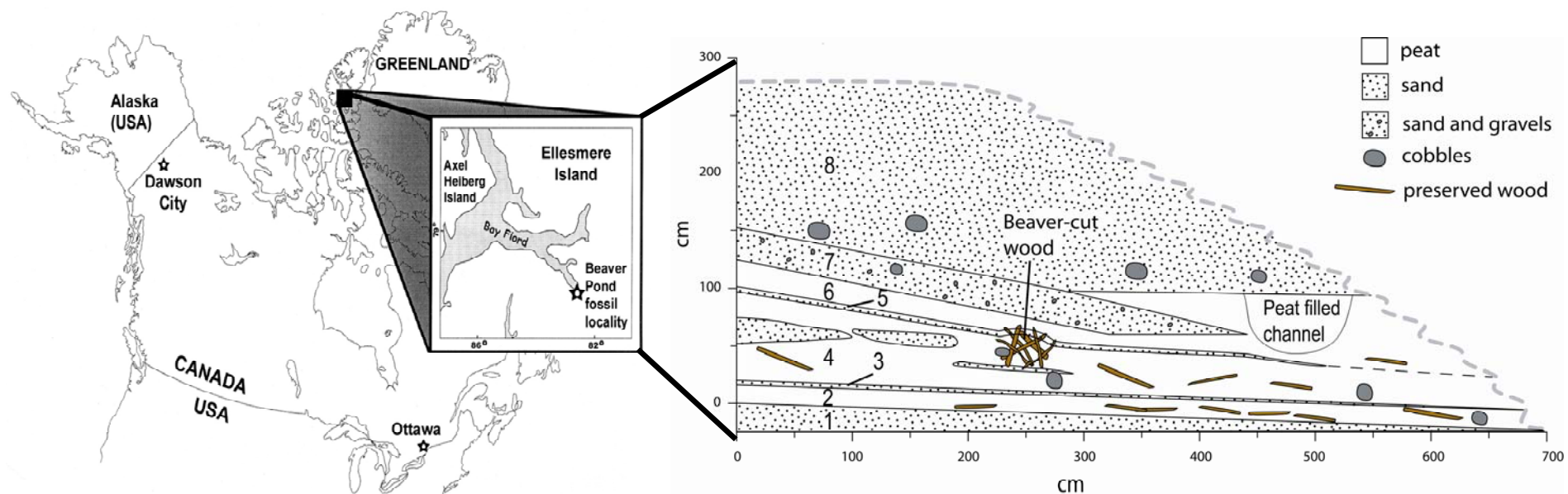
<b><u>Pliocene</u></b>	<u>Latitude</u>	<u>MAT (°C)</u>	<u>Reference</u>
	-85.1	-12.0	(Francis, 2007)
	-42.8	14.0	(Macphail, 1995)
	-35.2	14.0	(Macphail, 1997)
	-34.0	18.0	(Macphail, 1997)
	-17.4	18.0	(Kershaw, 1982)
	-16.7	22.0	(Macphail, 1997)
	10.0	27.0	(Graham, 1998)
	11.3	20.2	(Bonnefille, 2004)
	18.1	23.0	(Graham, 1997)
	19.4	24.5	(Graham, 1990)
	26.0	16.0	(Kou, 2006)
	27.4	26.4	(Willard, 1994b)
	33.1	7.0	(Iwauchi, 1994)
	35.2	23.0	(Fauquette, 1999)
	35.7	23.0	(Fauquette, 1999)
	36.4	21.0	(Fauquette, 1999)
	36.6	17.2	(Willard, 1994a)
	37.0	5.0	(Li, 2004)
	38.3	18.0	(Thompson, 1991)
	39.4	19.0	(Fauquette, 1999)
	40.5	17.0	(Mamedov, 1991)
	40.8	21.6	(Fauquette, 1999)
	41.2	18.3	(Fauquette, 1999)
	43.8	16.5	(Fauquette, 1999)
	44.6	12.3	(Grichuk, 1991)
	44.6	15.5	(Fauquette and Bertini, 2003 )
	45.8	13.0	(Wolfe, 1990)
	46.4	13.0	(Svetlitskaya, 1994)
	48.4	20.5	(Grichuk, 1991)
	51.0	11.1	(Mohr, 1984)
	51.0	14.5	(Utescher et al., 2000)
	51.8	9.0	(Ferguson and Knobloch,
	52.8	8.5	1998)
	55.1	9.8	(Grichuk, 1991)
	56.0	16.0	(Grichuk, 1991)
	56.1	8.5	(Volkova, 1991)
	58.2	9.8	(Grichuk, 1991)

58.2	5.7	(Willard, 1994a)
59.4	9.8	(Willard, 1994a)
60.0	2.0	(Pisareva, 2006)
64.1	2.5	(Fradkina 1991)
64.5	3.0	(Ager, 1994)
65.5	3.0	(Ager, 1994)
66.0	4.0	(Ager et al., 1994)
66.1	3.4	(Bennike and Boecher, 1990)
70.0	1.5	(Akhmetiev, (1991))
72.2	2.0	(Nelson and Carter, 1985)
78.3	-0.4	(Fradkina 1991)
78.3	-0.5	Present study
78.3	-0.6	Present study
78.3	-5.5	Present study
79.0	-4.1	(Ballantyne et al., 2006)
79.0	-7.3	(Elias and Matthews Jr, 2002)
79.0	-7.2	(Elias and Matthews Jr, 2002)
80.0	-8.7	(Elias and Matthews Jr, 2002)
82.0	-5.0	(Elias and Matthews Jr, 2002)
		(Elias and Matthews Jr, 2002)

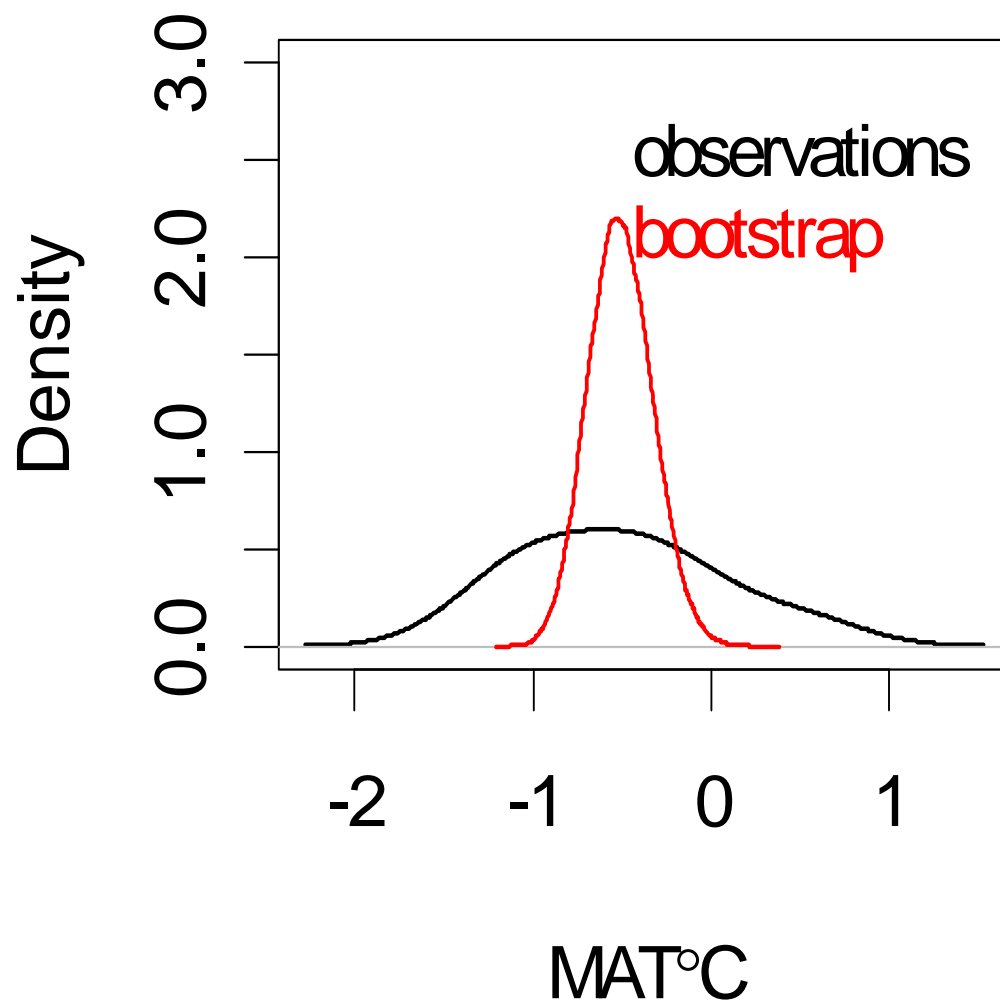
<b><u>Eocene</u></b>	-80.0	6.0	(Greenwood and Wing, 1995)
	-68.8	12.0	
	-62.8	15.0	
	-59.5	15.0	
	-57.0	15.0	
	-53.6	18.0	
	-53.2	18.0	
	-49.7	18.0	
	-48.4	20.0	
	-48.2	20.0	
	-45.4	20.0	
	-40.8	20.0	
	-30.5	23.0	
	-2.0	30.0	
	9.0	28.0	
	24.0	24.0	
	31.1	23.9	
	31.1	22.3	
	40.1	15.7	
	40.1	18.6	
	43.5	14.4	
	43.5	17.1	



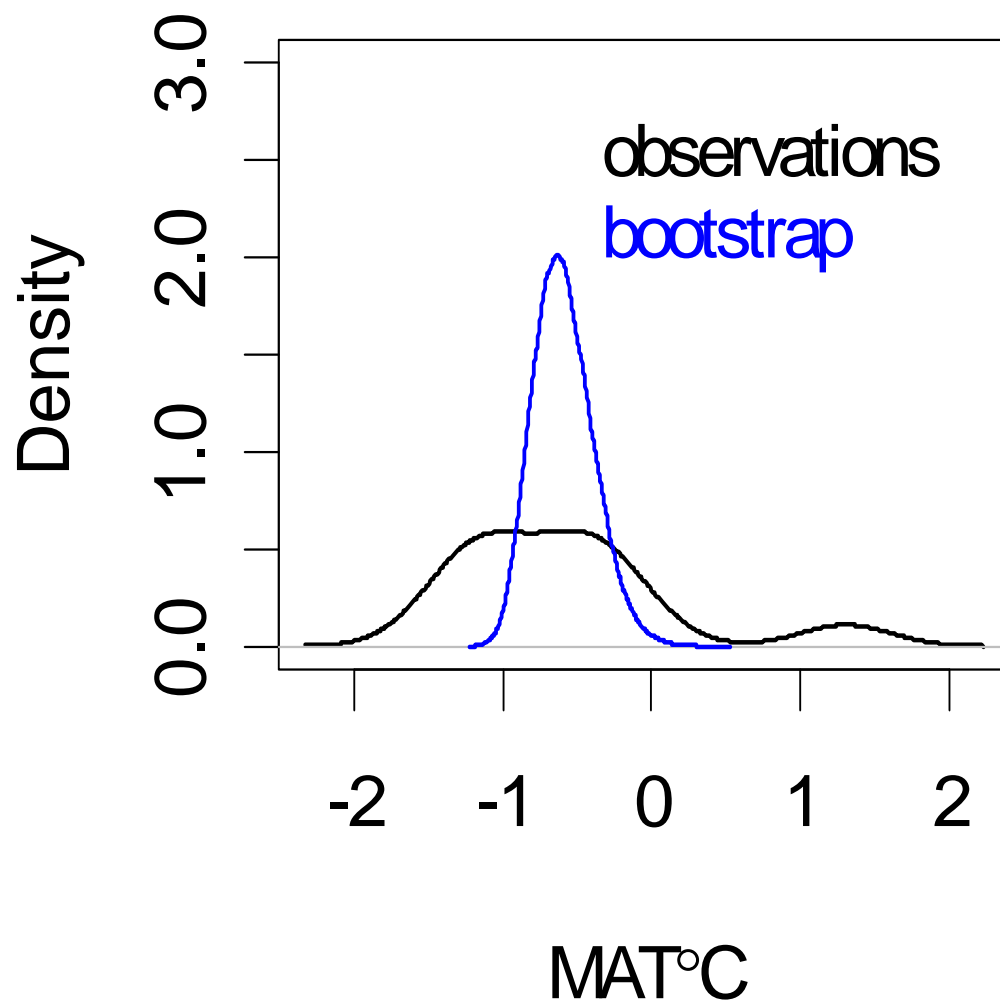
43.6	14.3
43.6	15.2
44.8	10.4
44.8	11.2
46.0	18.0
46.1	16.5
46.1	18.6
46.3	18.0
46.3	15.3
47.4	10.9
47.7	10.7
47.9	11.2
47.9	18.0
48.0	18.0
72.2	14.0
72.2	8.2
73.9	9.3
73.9	12.0



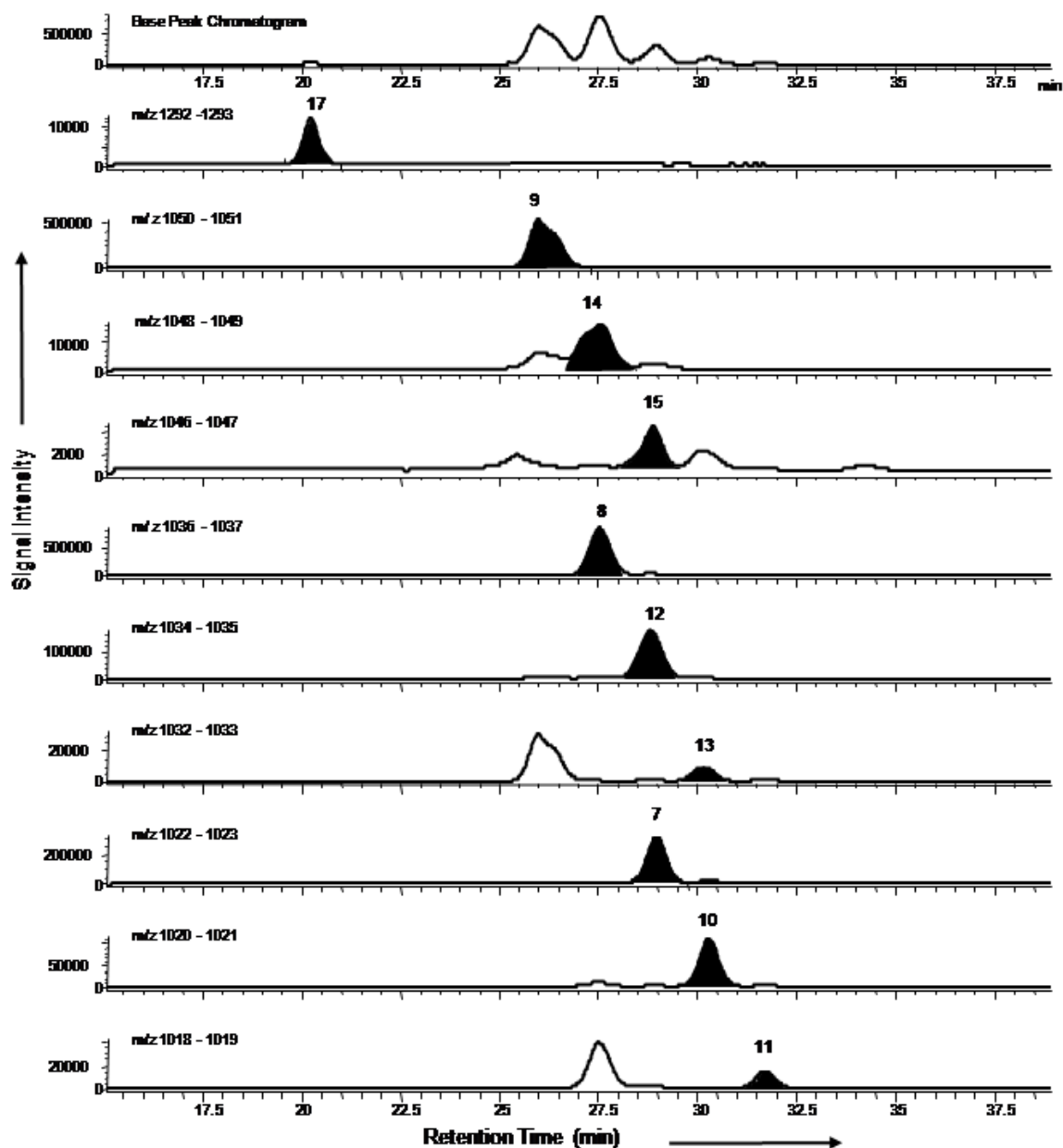
Supplementary Figure DR1. Location of sites used in this study. The Pliocene-aged Beaver Pond site from which fossils were collected is on Ellesmere Island (Nunavut). Modern tree samples used in the ring width and isotope analysis were from Ottawa (Ontario) and Dawson City (Yukon) (left). Cross-section of the Beaver Pond Site showing stratigraphic sections including Beaver-cut wood (Rybczynski, 2008) and peat layers (2-6) from which samples were taken for proxy analyses (right).



Supplementary Figure DR2. Probability density function of mean annual temperature (MAT) estimates for the Pliocene Arctic derived from the annual growth and oxygen isotopic composition of fossil trees. The probability density function for the observations is illustrated in black and bootstrapped estimates of the probability density function are illustrated in red.



Supplementary Figure DR3. Probability density function of mean annual temperature (MAT) estimates for the Pliocene Arctic derived from tetraethers present in palaeosol. The probability density function for the observations is illustrated in black and bootstrapped estimates of the probability density function are illustrated in blue.



Supplementary Figure DR4. Partial HPLC/MS Base Peak Chromatogram and mass chromatograms of the protonated molecule plus first isotope peak of the different GDGTs in one of the analyzed Beaver Pond peat samples collected from the middle of the section (Supp. Fig. 1) revealing the distribution of the branched GDGTs used to estimate MAT. Numbers identify peaks that refer to specific structures in the appendix of Weijers et al. (2006).

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