Supplement (part 1 of 2)

S1 Comparison of ASE and BD Methods

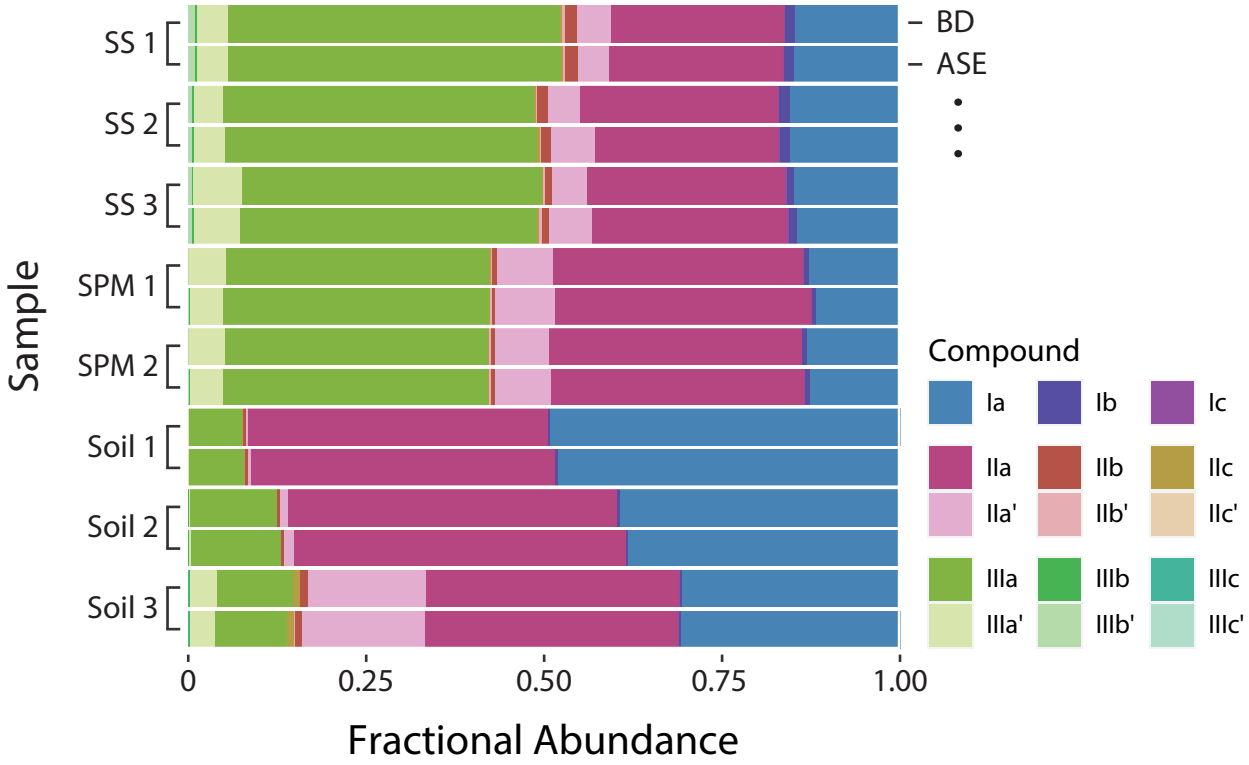

Figure S1. Comparison of fractional abundances (Full set) obtained using the Bligh and Dyer (BD; top of each pair) and accelerated solvent extractor (ASE; bottom of each pair) extraction methods. "SS" = Surface Sediment; "SPM" = Suspended Particulate Matter.

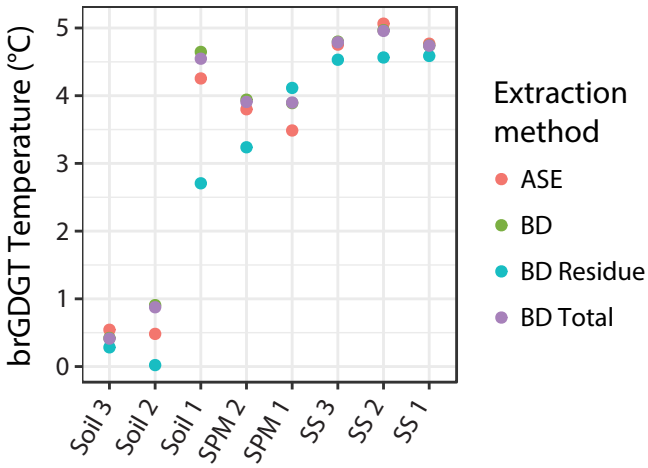

Figure S2. Comparison of brGDGT-derived temperatures obtained from samples extracted with the accelerated solvent extractor (ASE) and Bligh and Dyer (BD) extraction methods. The BD sample residue re-extracted with ASE ("BD Residue") and the sum of the BD and BD Residue samples ("BD Total") are additionally shown. Mean annual temperature for soil and lake samples were calculated using the Russell et al. (2018) and Naafs et al. (2017) MBT'$_{5Me}$ calibrations, respectively. "SS" = Surface Sediment; "SPM" = Suspended Particulate Matter.

S2 Comparison of WorldClim and logger temperatures

WorldClim- and iButton-derived climate normals were within one standard deviation for all months except June and July, for which the *in situ* logger temperatures were 1.0 ± 0.7 °C and 1.3 ± 1.0 °C higher, respectively. These warmer summer temperatures were most pronounced for sites sitting within deep glacial valleys, where high (> 100m) exposed rock walls may make them susceptible to warmer summer microclimates.

S3 Meth and MC Subset Variations

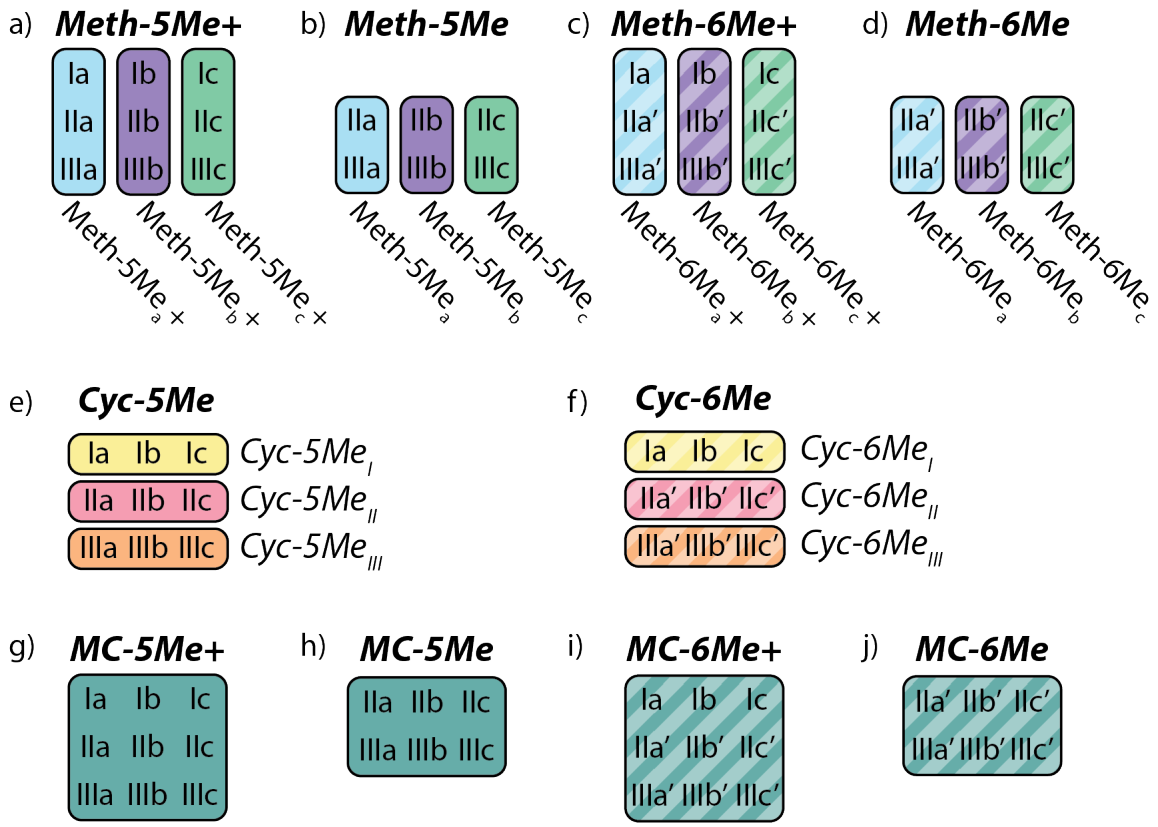

Figure S3. Structural subsets within the Meth (a-d), Cyc (e-f), and Meth-Cyc (MC; g-j) sets. Variations on the Meth and MC subsets that include (a, c, e, g) or exclude (b, d, f, h) tetramethylated compounds are shown. The complete Meth set (Fig. 2a) is composed of the Meth-5Me+ (a) and Meth-6Me (d) subsets. The complete MC set shown in (Fig. 2f) is composed of the MC-5Me+ (e) and MC-6Me (h) subsets.

Plots of the structural subset variations against MAF are additionally provided in the supplement_pt2.html file (Figs. S4-11).

S4 Structural Set Plots

Plots of all structural sets against MAF, conductivity, pH, and DO_{mean} are provided in the supplement_pt2.html file (Figs. S11-40).

S5 Calibration Results

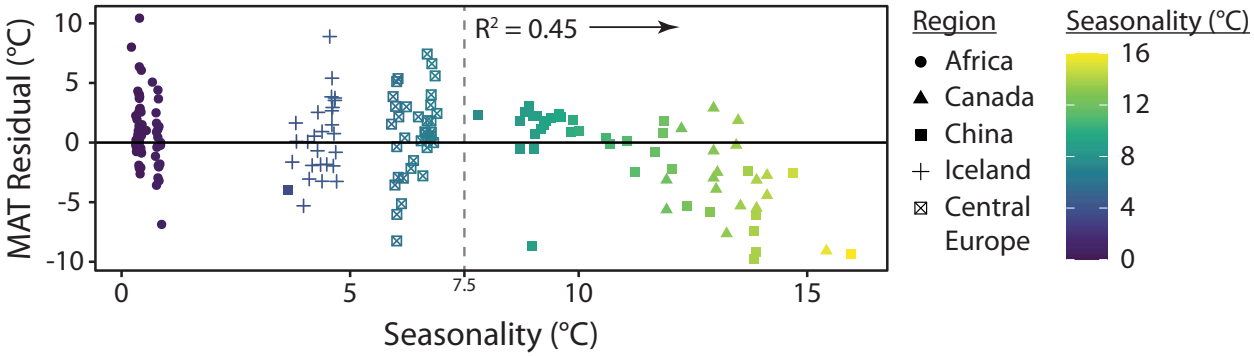

Figure S40. Residuals of the highest-performing fit (Meth set) for mean annual temperature (MAT) plotted against seasonality. The residuals correlate with seasonality (R2 = 0.14), especially when seasonality > 7.5 °C (R2 = 0.45).

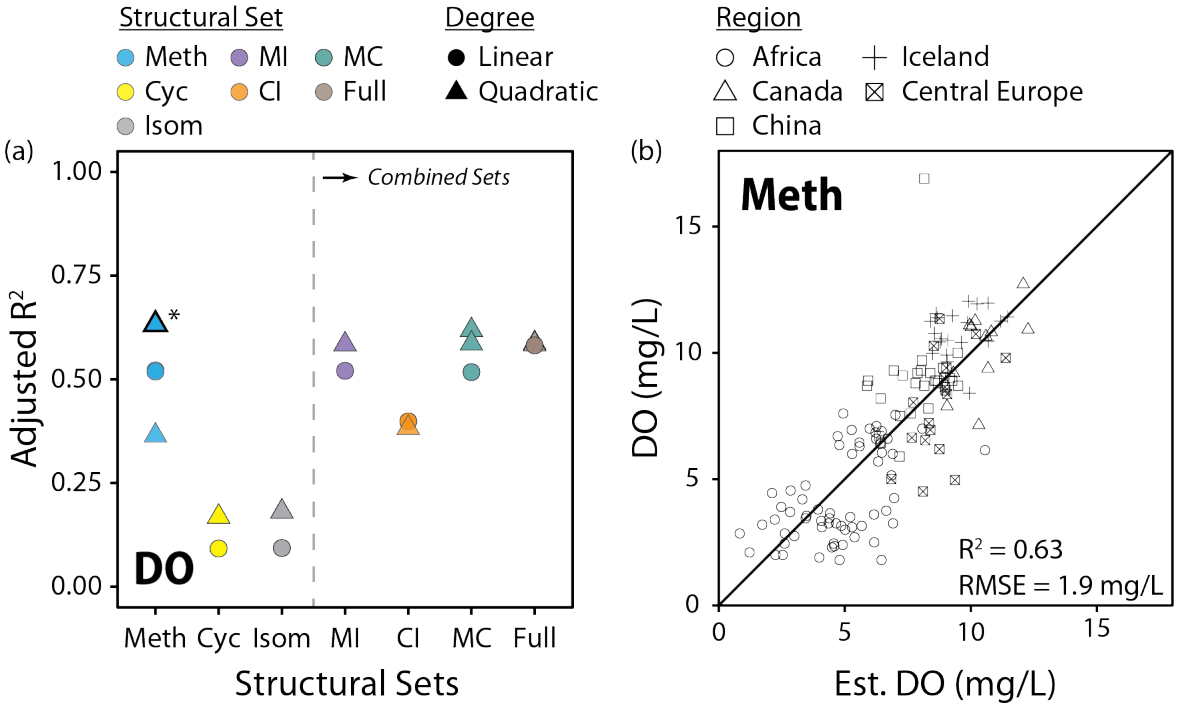

Figure S41. a) Performance (adjusted R2) of all linear and quadratic fits for mean lake water dissolved oxygen concentrations and brGDGT fractional abundances (FAs) calculated within the basic (Meth, Cyc, Isom; left of dashed line) and combined (Meth-Isom (MI), Cyc-Isom (CI), Meth-Cyc (MC), and Full sets; right of dashed line) structural sets. Results of both the SFS/SBE and combinatoric fitting methods are plotted. The highest performing fit (Meth set, quadratic, SFS/SBE) is bolded and marked with an asterisk in a) and plotted in b). "Est. DO" is the mean dissolved oxygen concentration estimated using this top fit.

Env. Variable	Subset	Adj. R^2	RMSE[a]	Variables	Compounds
MAF (°C)	**Full**	**0.91**	**1.97**	**6**	**15**
	MC	0.91	1.99	4	15
	MI	0.90	2.14	4	15
	Meth	**0.90**	**2.14**	**8**	**9**
	MI_a	0.90	2.18	4	5
	MBT'_{5Me}	0.89	2.32	1	7
	$Meth_a$	0.88	2.33	3	3
	$Meth_b$	0.79	3.10	5	5
	$Meth_c$	0.74	3.32	4	5
MST (°C)	Full	0.90	2.44	8	15
SWI (°C)	MC	0.89	30.13	6	15
WMT (°C)	Full	0.88	2.70	8	15
MAT (°C)	MI	0.87	3.44	9	15
ln(Conductivity)	**CI**	**0.83**	**0.66**	**7**	**15**
	MC	0.83	0.65	12	15
	Full	0.81	0.69	8	15
	MI	0.80	0.70	7	15
	Isom	0.76	0.78	6	8
	CI_{III}	0.75	0.80	3	6
	CI_{II}	0.73	0.84	4	6
	IR_{6Me}	0.66	0.95	1	12
	CI_I	0.65	0.95	4	3
pH	Full	0.74	0.55	8	15
	CI	**0.73**	**0.57**	**2**	**9**
	CI_{II}	0.68	0.62	3	6
	CBT	0.64	0.66	1	6
	CI_{III}	0.62	0.67	2	6
	CI_I	0.60	0.69	1	3
DO_{mean} (mg/L)	Meth	0.63	1.86	8	15
ln(Surface Area/Depth)	Full	0.59	2.26	7	15
ln(Volume)	Full	0.55	2.55	10	15
DO_{min} (mg/L)	Cyc_{III}	0.55	2.43	3	6
ln(Depth)	MI	0.35	1.02	6	15

Table S1. Summary of calibrations for all environmental variables (Env. Variable). "Variables" is the number of fitting variables used in each calibration. "Compounds" is the total number of compounds used in each calibration, including all those employed in fractional abundance calculations. Recommended fits are emphasized in bold. aRMSE units are indicated in the "Env. Variable" column.

In addition to those in the main text, we provide equations for the subset-specific $Meth_a$, $Meth_b$, and $Meth_c$ MAF fits:

$$MAF\ (°C) = 26.56(\pm 0.52) - 34.67(\pm 5.14) \times fIIa^2_{Meth} + 29.4(\pm 7.46) \times fIIIa^2_{Meth} - 49.43(\pm 4.92) \times fIIIa_{Meth}\ (n = 182, R^2 = 0.88, RMSE\ =\ 2.33°C) \quad (S1)$$

$$MAF\ (°C) = 79.72(\pm 21.83) + 103.96(\pm 19.08) \times fIb^2_{Meth} - 147.29(\pm 40.62) \times fIb_{Meth} - 80.14(\pm 23.81) \times fIIb^2_{Meth} - 7.19(\pm 1.98) \times fIIIb'^2_{Meth} - 89.73(\pm 26.54) \times fIIIb_{Meth} (n = 182, R^2 = 0.79, RMSE = 3.10°C) \quad (S2)$$

$$MAF\ (°C) = 9.38(\pm 1.97) + 19.92(\pm 2.41) \times fIc^2_{Meth} - 8.44(\pm 2.89) \times fIIc_{Meth} + 18.83(\pm 5) \times fIIIc'^2_{Meth} - 18.4(\pm 3.32) \times fIIIc'_{Meth} (n = 157, R^2 = 0.74, RMSE = 3.32°C) \quad (S3)$$

We also provide the subset-specific CI_I, CI_{II}, and CI_{III} fits for conductivity,

$$ln(Cond.) = -20.07(\pm 4.33) + 22.13(\pm 4.34) \times fIa^2_{CI} - 61(\pm 10.22) \times fIb^2_{CI} + 73.24(\pm 10.93) \times fIb_{CI} + 123.55(\pm 26.57) \times fIc^2_{CI} (n = 143, R^2 = 0.65, RMSE = 0.95) \quad (S4)$$

$$ln(Cond.) = 7.11(\pm 0.82) + 5.17(\pm 1.97) \times fIIa^2_{CI} - 9.42(\pm 2.25) \times fIIa_{CI} - 38.77(\pm 14.13) \times fIIb'^2_{CI} + 14.36(\pm 4.83) \times fIIb'_{CI} (n = 143, R^2 = 0.73, RMSE = 0.84) \quad (S5)$$

$$ln(Cond.) = 7.46(\pm 0.24) - 5.28(\pm 0.32) \times fIIIa_{CI} - 268.59(\pm 82.68) \times fIIIb^2_{CI} + 31.22(\pm 7.74) \times fIIIb_{CI} (n = 143, R^2 = 0.75, RMSE = 0.80) \quad (S6)$$

and for pH,

$$pH = 5.96(\pm 0.12) + 7.68(\pm 0.5) \times fIb_{CI} (n = 154, R^2 = 0.60, RMSE = 0.69) \quad (S7)$$

$$pH = 8.31(\pm 0.25) - 3.37(\pm 0.28) \times fIIa_{CI} - 23.45(\pm 7.59) \times fIIb^2_{CI} + 10.34(\pm 2.38) \times fIIb_{CI} (n = 154, R^2 = 0.68, RMSE = 0.62) \quad (S8)$$

$$pH = 8.41(\pm 0.13) - 2.84(\pm 0.22) \times fIIIa^2_{CI} + 7.48(\pm 2.37) \times fIIIb_{CI} (n = 154, R^2 = 0.62, RMSE = 0.67) \quad (S9)$$

Finally, we provide the highest-performing DO_{mean} calibration:

$$DO_{mean}\ (mg/L) = 7.6(\pm 2.32) - 12.03(\pm 2.6) \times fIa^2_{Meth} - 2.1(\pm 0.74) \times fIc^2_{Meth} - 28.66(\pm 8.5) \times fIIIa'^2_{Meth} + 31.09(\pm 8.66) \times fIIIa'_{Meth} + 36.85(\pm 8.37) \times fIIIa^2_{Meth} - 35.89(\pm 7.93) \times fIIIa_{Meth} - 15.29(\pm 3.44) \times fIIIb'^2_{Meth} + 15.82(\pm 2.55) \times fIIIb'_{Meth}\ (n = 140, R^2 = 0.63, RMSE\ =\ 1.86\ mg/L) \quad (S10)$$

Other calibration equations are available upon request.