Supporting Online Material for

Biomarker Evidence for Photosynthesis During Neoproterozoic Glaciation

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Analytical Methods:

Samples were processed serially, rather than in parallel, to avoid cross-contamination. About 30 g of rock was washed then sonicated in distilled water for ~10 s. Samples were air-dried at room temperature and crushed into <5 cm pieces with a jaw-type rock crusher that had been cleaned 4x each with acetone then dichloromethane (DCM). These large pieces were then sonicated with ~250 ml 9:1 DCM:MeOH for 2 minutes. Solvent was collected and rock pieces were crushed to <1 cm on a smaller rock crusher cleaned as before. Ultrasonic extraction was repeated, and the sample was powdered in a shatterbox that was cleaned by grinding quartz sand followed by 4x acetone and DCM cleaning.

The powdered rock was extracted in a microwave-accelerated reaction system (MARSXpress): 20 g of rock was split equally between 5 clean Teflon vessels, 25 ml of 9:1 DCM/MeOH was added to each vessel, and the samples were extracted at 100°C for 15 minutes with stirring. Extracts were filtered through combusted glass-fiber filters to remove particulates, and solvent was evaporated to ~30ml under nitrogen at 35°C, taking care not to allow samples to completely dry. Elemental sulfur was removed by filtration through activated copper (~3.5g, -40+100 mesh), and the S°-free extract was evaporated to near-dryness under N_2. Extract was transferred to a vial with hexane, solvent volume was reduced under N_2 to 100µl, and then analyzed by gas chromatography/mass spectrometry (GC/MS). Extracts from the two preliminary extractions were prepared and analyzed following the same procedures. All samples were analyzed on a ThermoFinnigan Trace GC-DSQ quadrupole MS equipped with a DB-5MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness). 1 µl aliquots were injected into a PTV injector (35°C hold for 3 min, 14.5°C/s to 200°C then 12°C/s to 350°C with a 3 minute hold). The column oven was programmed at 20°C/min to 130°C, then 5°C/min to 320°C with a 20 minute hold.

After initial GC/MS analyses, each of the final extracts was separated into fractions by column chromatography. Polar compounds (mainly phthalates and other plasticizers) were first separated from hydrocarbons on 1.0g silica gel (100-200 mesh, 5% deactivated) dry-packed into Pasteur pipettes. Hydrocarbons were eluted with 3.75 ml 8:2 Hexane:DCM (F1) and polar compounds were eluted with 6ml 7:3 DCM:MeOH (F2). Saturates and aromatics were then separated on silica gel with 10% $AgNO_3$ dry-packed into Pasteur pipettes. Aliphatic compounds were eluted with 5ml hexane (F1a) and aromatic compounds were eluted with 4ml DCM (F1b). The three fractions (polar, aromatic, aliphatic) were concentrated under N_2, and analyzed by GCMS using the same conditions as above.

Laboratory blanks of the solvents, copper, silica gel, silver-impregnated silica gel and MARS vessels were analyzed, and a block of pre-baked basalt was spiked with a standard lipid solution and then subjected to the entire analytical procedure. No hydrocarbons, oil residues or UCM were observed in any of the blanks. Biomarker yields were confirmed with replicate extractions of several samples. Biomarker identities were confirmed by metastable-reaction monitoring GCMS analyses of split aliquots from our extracts by E. Grosjean and R. Summons (Massachusetts Institute of Technology). TOC abundances were measured by a Eurovector elemental analyzer in line with an Isoprime

gas source isotope ratio mass spectrometer (University of Maryland) and also by Dumas combustion.

Figure S1: Selected-ion GC/MS chromatograms for the eight samples showing distributions of hopanes (left; m/z 191), steranes (middle; m/z 217) and alkylated 2,3,6-trimethylbenzenes (right; m/z 133). For each column, chromatograms are depicted at the same scale; the mass spectrum of the compound marked with a dot is shown in Figure S2.

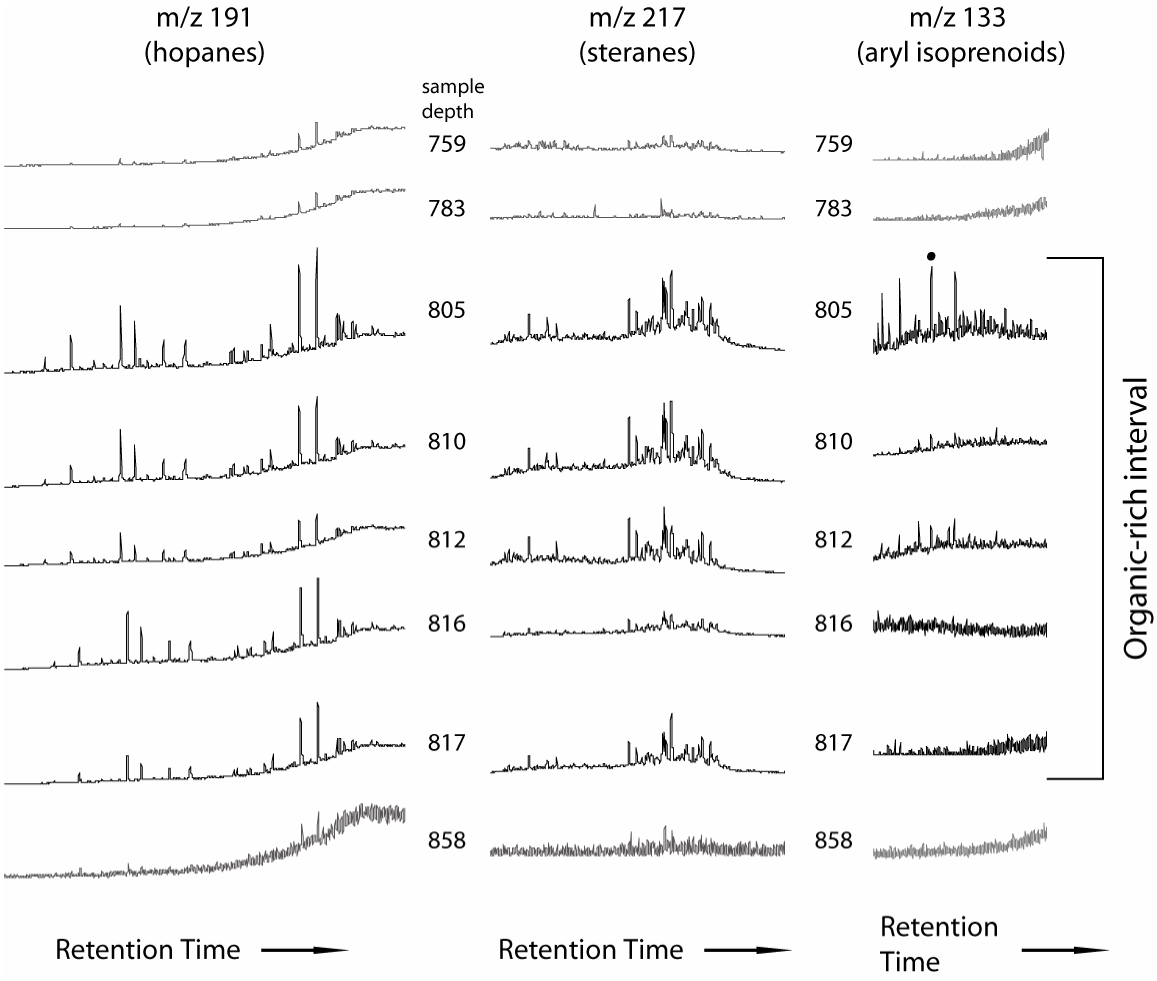

Figure S2: The mass spectrum of the alkylated 2,3,6-trimethylbenzene indicated in Figure S1. For comparison, the NIST mass spectrum for 1-(3-methylbutyl)-2,3,6-trimethylbenzene, a shorter-chain alkylated 2,3,6-trimethylbenzene, is shown in inset (NIST #245506).

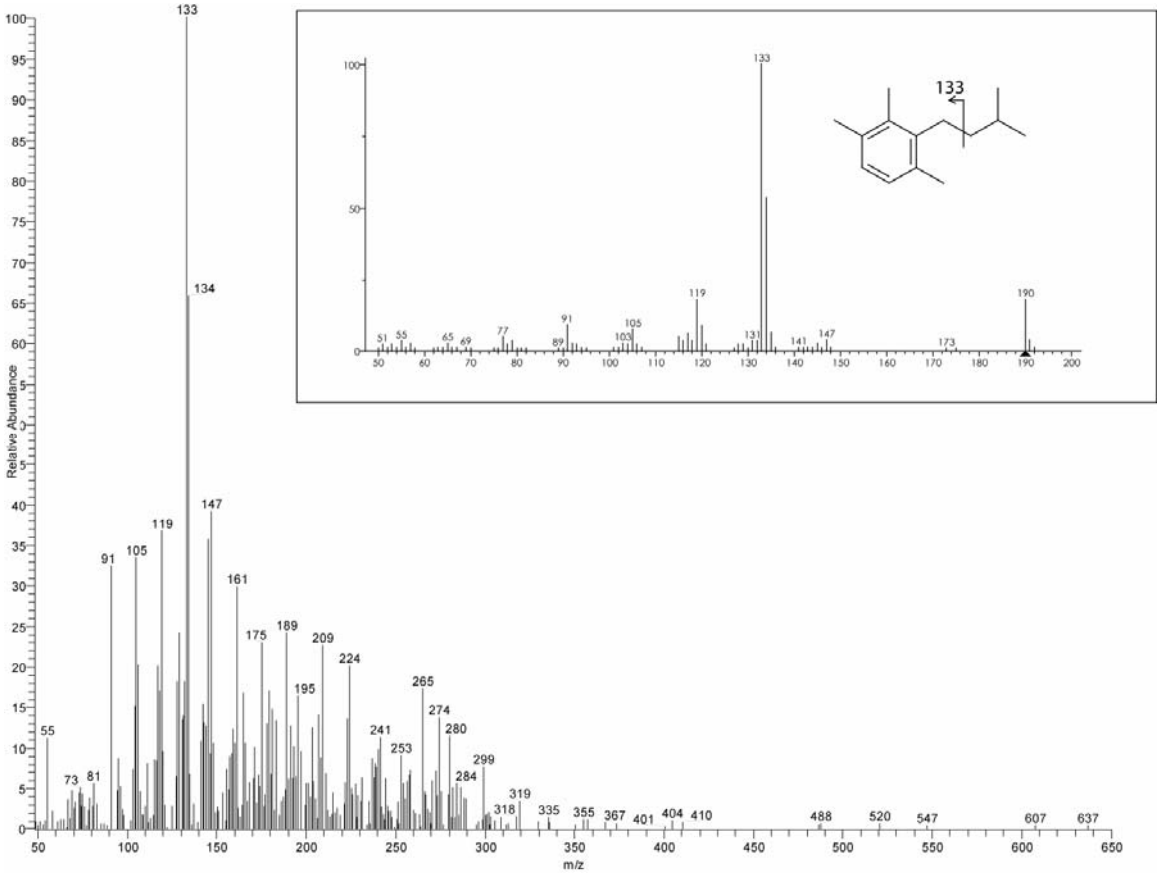

Table S1: Kerogen and bitumen contents of samples analyzed for biomarker content.

sample depth (m)	Lithology	$CaCO_3$ (%)	C_{org} (%)	H/C of kerogen	Extract yield (µg/g)	$\delta^{13}C$ kerogen (‰)	$\delta^{13}C$ bulk extract (‰)
759	rhythmic marls	8	0.11	0.23	5.0	-25.7	-24.1
783	rhythmic marls	43	0.08	0.10	7.7	-29.1	-23.7
805	black shale	6	0.43	0.11	8.1	-25.1	-25.3
810	black shale	6	0.44	0.54	4.8	-26.0	-25.6
812	black shale	8	0.69	0.06	10.8	-24.9	-24.7
816	black carbonate	91	0.86	0.97	5.8	-22.6	-23.4
817	black shale	3	1.01	0.04	13.5	-26.3	-24.2
858	diamictite	95	0.01	0.09	2.4	-21.5	-24.5

Table S2: Biomarker ratios in samples from organic-rich black shale samples in core MAF 42-88. Ratios were measured using standard methods (*S1*). The first six ratios reflect the thermal maturity of the organic matter, comparing the relative abundances of the biomarkers' biologic form (e.g, 20R steranes) to the isomers created by thermal alteration (e.g., 20S steranes). The last seven ratios reflect changes in biologic input, comparing the relative abundances of biomarkers from different organisms (e.g., steranes to hopanes). The two numbers for sample 805 m represent replicate determinations of the biomarker ratios.

	Depth	**805**	**810**	**812**	**816**	**817**
MATURITY PARAMETERS	C_{27} Ts/(C_{27} Ts+ C_{27}Tm)	0.37/0.37	0.42	0.58	0.40	0.39
	C_{29}TS/ C_{29} hopane	0.17/0.18	0.16	0.24	0.19	0.20
	(Ts+Tm)/C_{29} hopane	0.61/0.61	0.62	0.98	0.58	0.64
	Diasterane/sterane	0.42/0.42	0.52	0.88	0.47	0.45
	C_{29} sterane 20S /(20S+20R)	0.49/0.49	0.53	0.55	0.50	0.51
	C_{29} sterane αββ/(ααα+αββ)	0.47/0.47	0.51	0.54	0.46	0.50
SOURCE PARAMETERS	C_{27} sterane /(C_{27} +C_{28}+C_{29} steranes)	0.50/0.50	0.50	0.46	0.45	0.35
	C_{28} sterane /(C_{27} +C_{28}+C_{29} steranes)	0.21/0.21	0.20	0.21	0.25	0.27
	C_{29} sterane /(C_{27} +C_{28}+C_{29} steranes)	0.29/0.29	0.30	0.33	0.30	0.39
	C_{29} hopane/C_{30} hopane	1.03/1.02	1.04	1.02	1.05	1.09
	C_{30} hopane/C_{31} hopane	1.73/1.74	1.65	1.69	1.71	1.81
	2α-Me ratio (%)	9.0/9.0	8.5	9.6	10.8	8.5
	Sterane/hopanes	0.54/0.54	0.67	1.25	0.68	0.63

Table S3: Concentrations (pg/g) of selected biomarkers found in core MAF 42-88. Biomarker concentrations were determined using peak areas from metastable-reaction monitoring GCMS chromatograms versus a coinjected standard (50ng D4 24-ethylcholestane).

Depth	**805**	**810**	**812**	**816**	**817**
2α-Me hopanes	1.98	1.36	1.30	1.35	2.96
Cholestane (C_{27})	18.27	16.93	27.06	11.77	24.89
Ergostane (C_{28})	7.74	6.69	12.46	6.40	19.09
Stigmastane (C_{29})	10.78	10.30	19.64	7.80	27.98
C_{29} Hopane	20.77	15.17	12.49	11.63	34.82
C_{30} Hopane	20.13	14.58	12.28	11.13	31.96
C_{31} Hopane	11.60	8.81	7.28	6.52	17.65
Gammacerane	2.00	0.38	0.56	1.05	2.63
3β-Me hopanes	0.73	0.15	0.62	0.52	0.95

References and Notes:

S1. J. J. Brocks, G. A. Logan, R. Buick, R. E. Summons, *Science* **285**, 1033 (1999).