Supporting Information

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SI Text

Effects of Extraction Method on HG Recovery. The relative extraction efficiencies of HGs using three different extraction procedures were determined, i.e., ultrasonic extraction with DCM:MeOH (2:1; ×4), Bligh and Dyer extraction (1), and accelerated solvent extraction (ASE). Roughly equal amounts (ca. 1.5 g) of surface sediments from the North Sea barrier island Schiermonnikoog (The Netherlands) were extracted with these three techniques and identical amounts of the resulting HG fractions were injected on the HPLC/MS-MS allowing comparison of the relative amounts of HGs obtained by each extraction technique. The measured relative signal intensities per gram sediment for the individual heterocyst glycolipids of ASE extraction and ultrasonic extraction were normalized to those of the Bligh and Dyer procedure (Fig. S4). These analyses revealed that, despite the high pressure and temperature, ASE extraction yields similar or even higher recovery of HGs than the generally used procedure for IPL (Intact Polar Lipid) analysis, i.e., the Bligh and Dyer extraction. We then tested the general stability of IPLs under ASE conditions by extracting cyanobacterial biomass, containing intact polar lipids with glycosidic and phosphate head groups (Fig. S5). This experiment showed that heterocyst glycolipids are stable under ASE conditions but that the other IPLs decrease substantially using this technique. Hence, ASE extraction is a suitable technique to investigate HGs in ancient sediments.

1. Rütters H, Sass H, Cypionka, H, and Rullkötter, J. (2002) Phospholipid analysis as a tool to study complex microbial communities in marine sediments. *J. Microbiol. Meth.* 48:149–160.

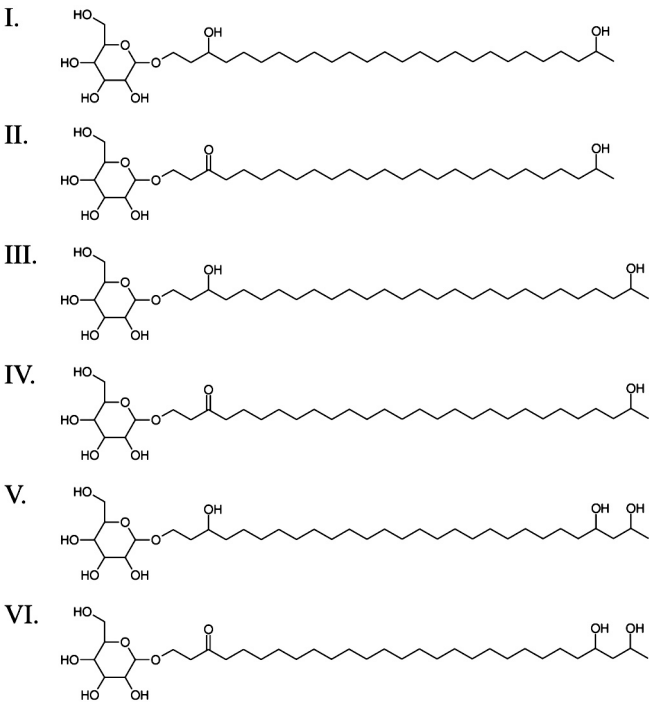

Fig. S1. Structures of heterocyst glycolipids detected in cultures and environmental samples.1-(O-hexose)-3,25-hexacosanediol (*I*); 1-(O-hexose)-3-keto-25-hexacosanol (*II*);1-(O-hexose)-3,27-octacosanediol (*III*); 1-(O-hexose)-3-keto-27-octacosanol (*IV*); 1-(O-hexose)-3,25,27-octacosanetriol (*V*) and 1-(O-hexose)-3-keto-25,27-octacosanediol (*VI*).

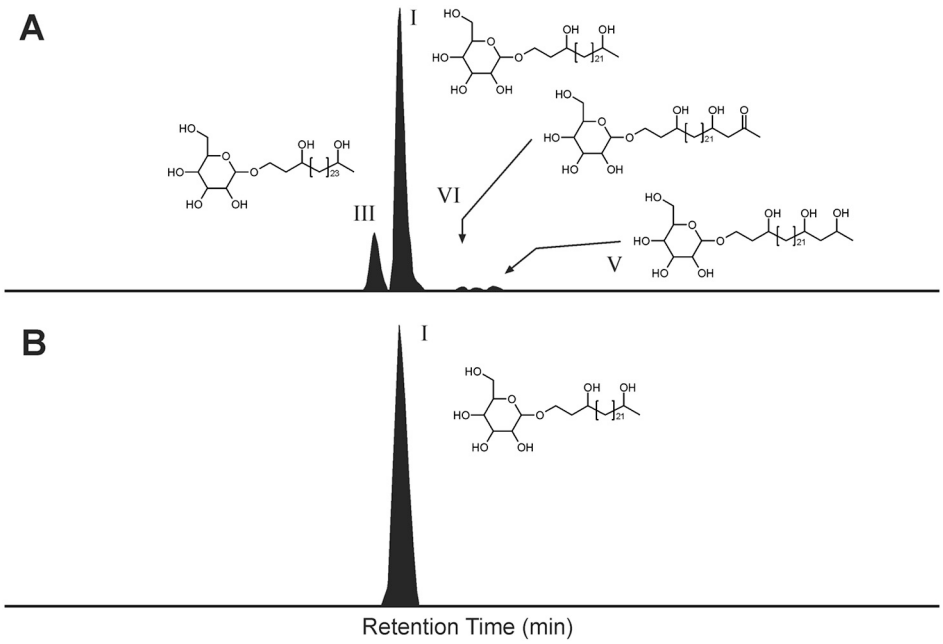

Fig. S2. HPLC/MS-MS summed ion chromatograms of selective reaction monitoring (SRM) transitions of glycolipids in extracts of sediments from the (*A*) Oligocene Lake Enspel and (*B*) Eocene Lake Messel. Note that the 47 million year old sediments of Lake Messel only contained 1-(O-hexose)-3,25-hexacosanediol (*I*), which was also the only HG detected in the Eocene Green-River Formation. In contrast, the Oligocene sediments of Lake Enspel are characterized by six different HGs (see Table S1).

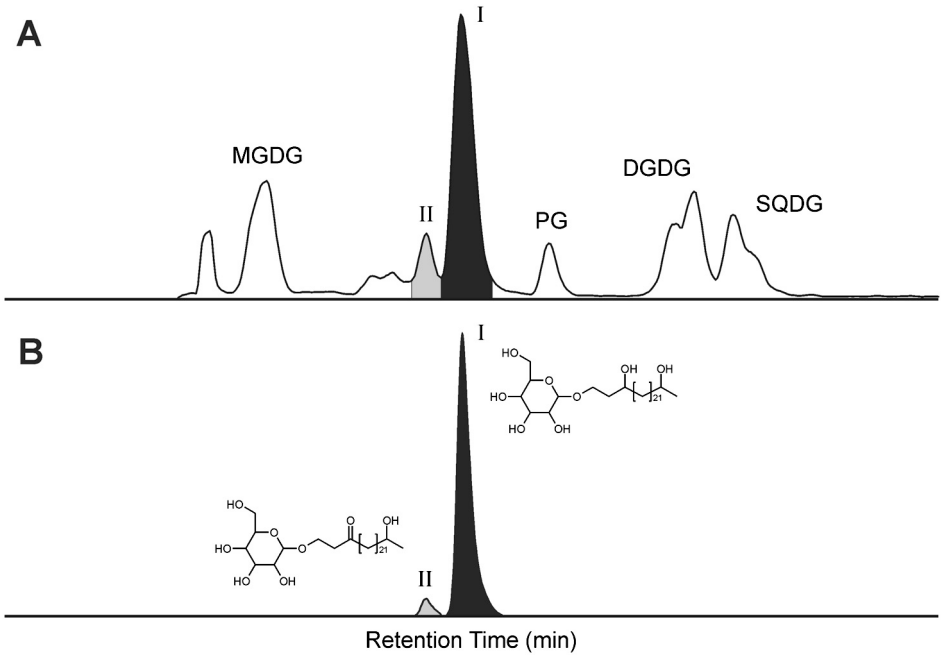

Fig. S3. Comparison of HG distributions in *Anabaena* CCY9613 using (*A*) base peak chromatogram of full scan analysis and (*B*) summed ion chromatograms of SRM transitions. MGDG = monoglycoside diacylglycerols, DGDG = diglycoside diacylglycerols, PG = phosphoglycerol diacylglycerols, SQDG = sulfoquinosovyl diacylglycerides.

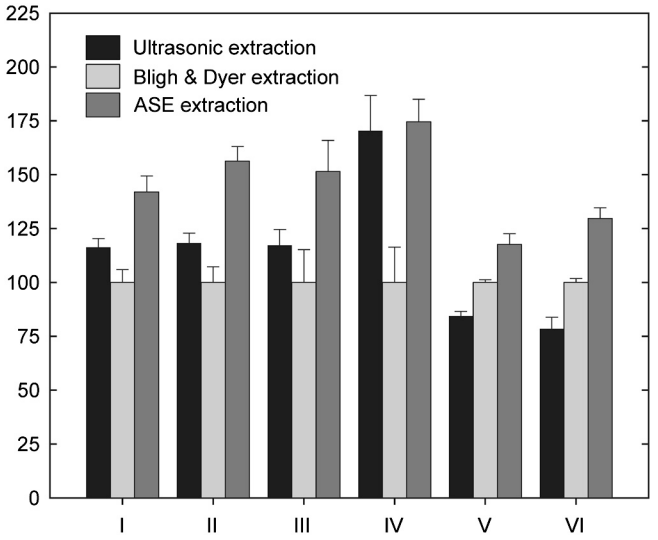

Fig. S4. Effect of different extraction procedures on the relative heterocyst glycolipid recovery. ASE and ultrasonic extraction are normalized to extraction yields from the Bligh and Dyer procedure. Note that ASE yielded highest extraction efficiencies. Roman numerals refer to structures presented in Fig. S1.

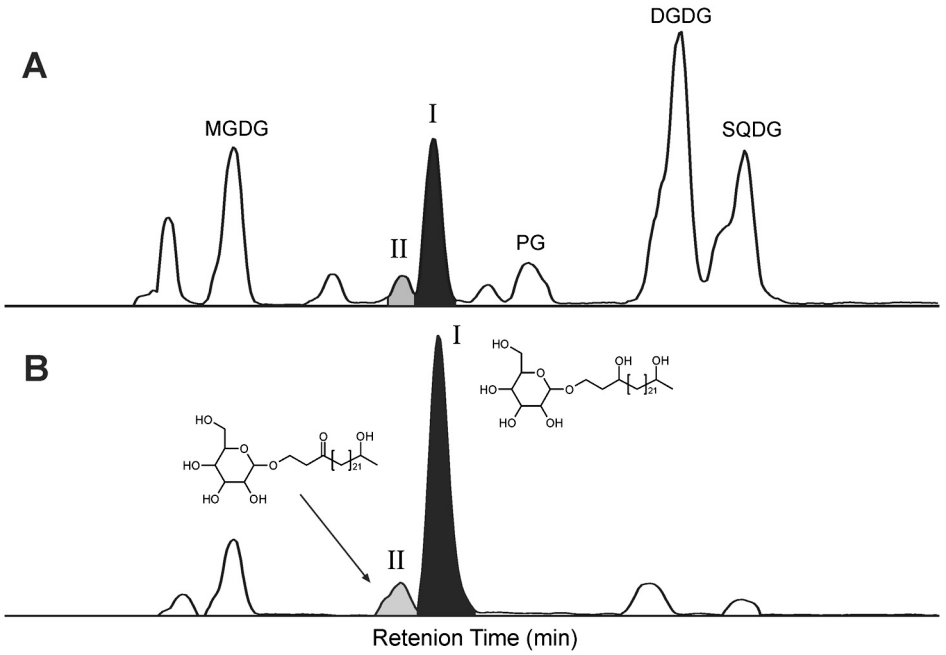

Fig. S5. Base peak chromatograms of full scan analysis of total lipid extracts of *Nostoc* CCY0012 extracted using (*A*) a modified Bligh and Dyer extraction procedure (1) and (*B*) the ASE technique. Note that the diacylglycerides with phosphor polar head groups, and to a lesser extent those with a (sulfur) sugar head group, are readily degraded upon ASE extraction, while heterocyst glycolipids are seemingly not affected by the rigorous extraction procedure. MGDG = monoglycosyl diacylglycerides, DGDG = diglycosyl diacylglycerides, PG = phosphoglycerol diacylglycerides, SQDG = sulfoquinosovyl diacylglycerides.

Table S1. Relative distribution of individual HGs as a percentage of total HG lipids in various cyanobacterial cultures, the freshwater fern *Azolla filiculoides* and contemporary sediments (Roman numerals refer to structures in Fig. S1)

Cultures	Types	Strain	I	II	III	IV	V	VI
Anabaena sp.	Nostocaceae	CCY9402*	0.4	0.1	83	16	-	-
Anabaena sp.	Nostocaceae	CCY9613	95	5	0.3	-	-	-
Nostoc sp.	Nostocaceae	CCY0012*	97	3	0.2	0.1	-	-
Calothrix sp.	Rivulariaceae	CCY9923*	0.2	0.4	2	1	61	36
Azolla filiculoides	Fern		84	7	9	-	-	-
Lake/sea	Locations	Depth/type						
Baltic Sea	Landsort deep	Sed. (2 mbsf)	49	25	2	1	9	14
		Sed. (34 mbsf)	77	8	4	1	8	2
Black Sea		Sed. (27 mbsf)	93	5	2	0.3	-	-
Kyllaren	Norway	Surface sed.	47	40	5	8	-	-
Schiermonnikoog	The Netherlands	Intertidal flat	90	1	0.5	0.1	8	0.4
Lake Ohrid	Macedonia	Surface sed.	10	1	66	23	-	-
Lake Malawi	Malawi	Surface sed.	11	0.1	86	3	-	-
Lake Tanganyika	Dem. Rep. Congo	Surface sed.	92	0.8	7	0.2	-	-
Lake Keilambete	Australia	Surface sed.	99	0.3	0.6	0.1	-	-
Lake Challa	Kenya	POM†	99	<1	<1	<1	<1	<1
		Sed. trap	91	4	3	1	<1	-
		Sediment	64	22	2	12	-	-

*Data from Bauersachs et al. (1).
†POM = Particulate organic matter.

1 Bauersachs T, Compaoré J, Hopmans EC, Stal LJ, Schouten S, Sinninghe Damsté JS (2009) Distribution of heterocyst glycolipids in cyanobacteria. *Phytochem.* 70:1370–1376.

Table S2. Relative distribution of individual HGs as a percentage of total HG lipids in a number of ancient sediments (Roman numerals refer to structures presented in Fig. S1)

Location		Age	I	II	III	IV	V	VI
Lake Enspel,	Germany	Oligocene	22	1	42	27	6	2
Lake Messel,	Germany	Eocene	10,000	-	-	-	-	-
Green-River Formation,	USA	Eocene	100	-	-	-	-	-
Mediterranean sapropel S1		Pleistocene	12	-	86	2	-	-
Mediterranean sapropel S5		Pleistocene	22	1	75	2	-	-
ACEX core 11X		Eocene	96	4	-	-	-	-

Table S3. SRM settings for detection of heterocyst glycolipids

Heterocyst glycolipids	$[M + H]^+$ m/z	Product m/z	Collision energy (V)
1-(O-hexose)-3,25-hexacosanediol (*I*)	577.5	379.5	15
		415.5	10
1-(O-hexose)-3-keto-25-hexacosanol (*II*)	575.5	377.5	15
		395.5	14
1-(O-hexose)-3,27-octacosanediol (*III*)	605.4	407.5	15
		443.5	10
1-(O-hexose)-3-keto-27-octacosanol (*IV*)	603.5	405.5	15
		423.5	14
1-(O-hexose)-3,25,27-octacosanetriol (*V*)	621.6	387.5	22
		405.5	18
		423.5	17
1-(O-hexose)-3-keto-25,27-octacosanediol (*VI*)	619.6	403.5	18
		421.5	16
		439.5	11