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Supplement of

Sterol preservation in hypersaline microbial mats

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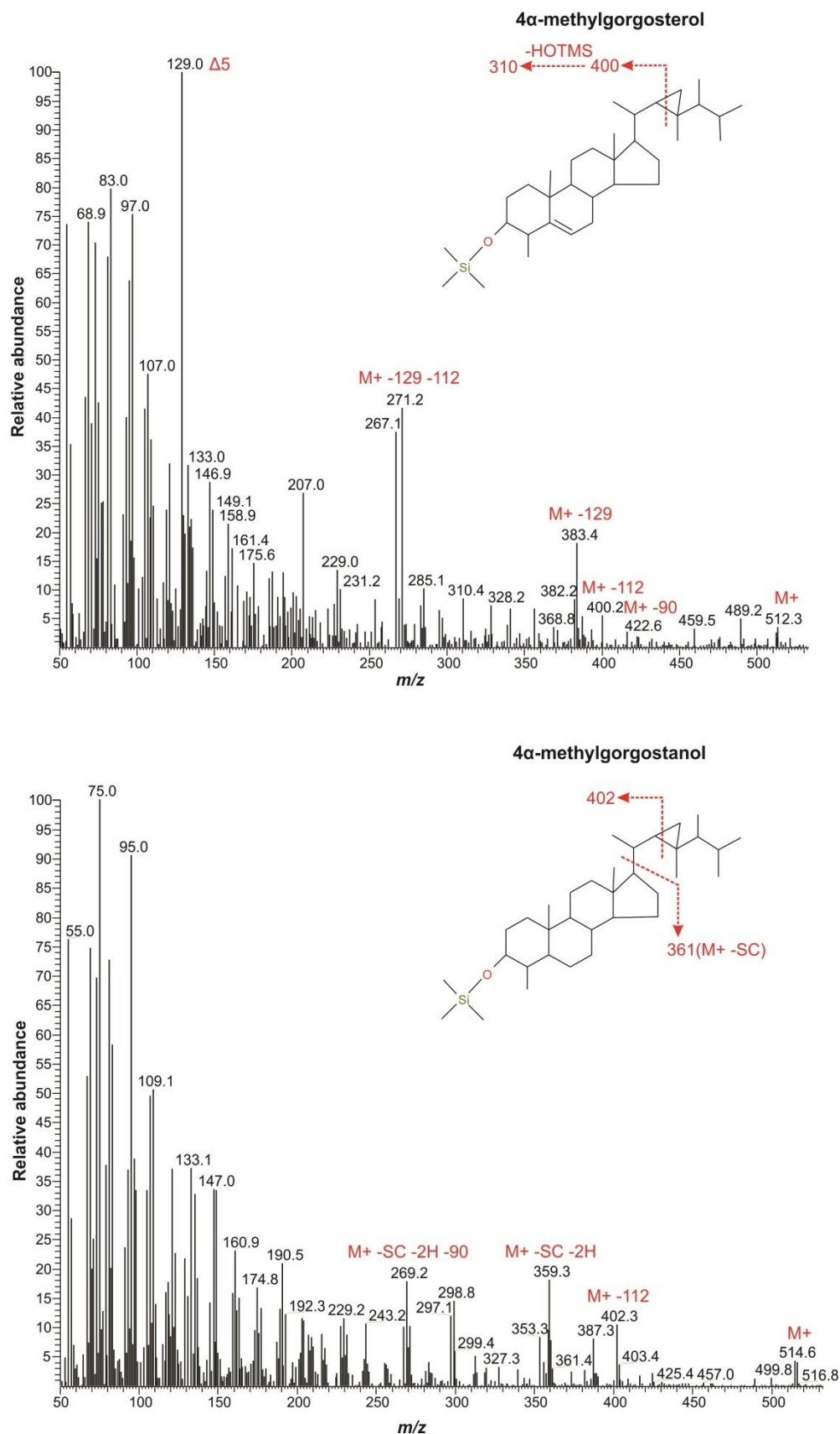


Figure S1. Mass spectra of 22,23-methylene-4 α ,23,24-trimethylcholest-5-en-3 β -ol (4 α -methylgorgosterol) and 22,23-methylene-4 α ,23,24-trimethylcholestan-3 β -ol (4 α -methylgorgostanol) observed in the studied microbial mat (Atwood et al., 2014; Houle et al., 2019).

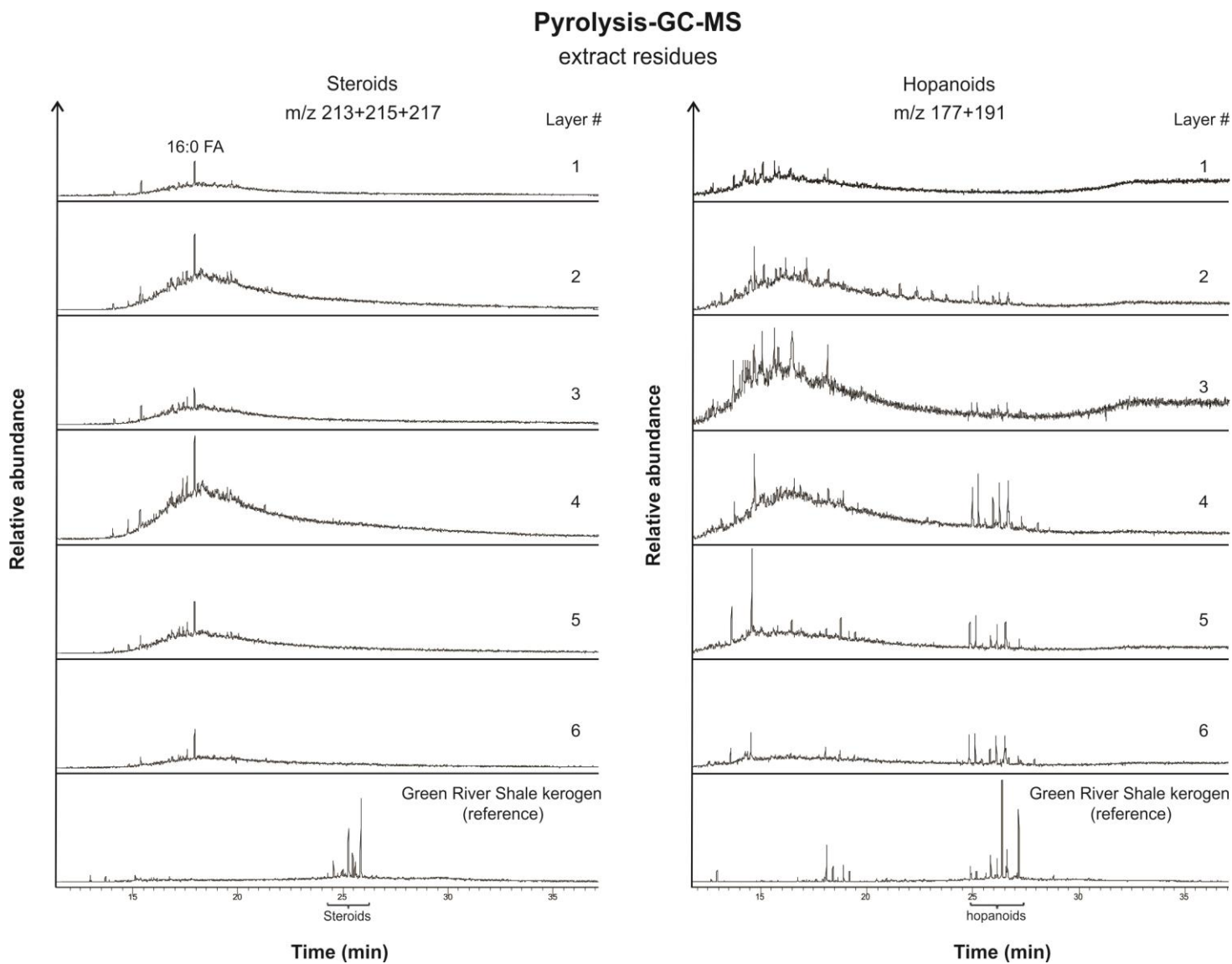


Figure S2. Pyrolysis-GC-MS ion chromatograms showing the distributions of steroids (m/z 213+215+217) vs. hopanoids (m/z 177+191) in the insoluble organic matter of the microbial mat layers (extraction residues after decalcification; Eocene Green River Shale for reference: Eastern Utah, White River Mine, BLM Oil Shale Research, Development, and Demonstration Lease UTU-84087).

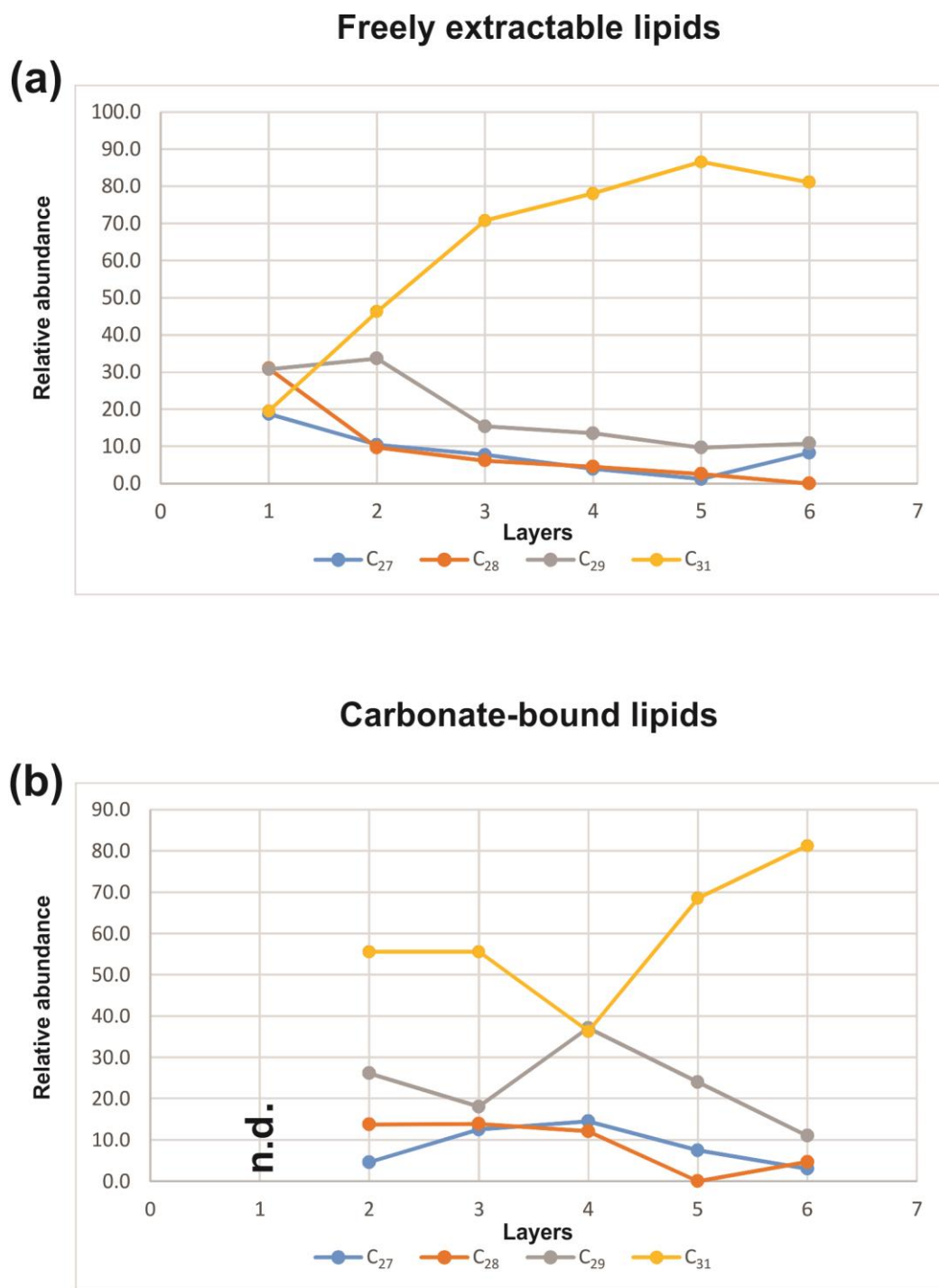


Figure S3. Distributions of summed C₂₇- vs. C₂₈- vs. C₂₉- vs. C₃₁-sterols in the (a) freely extractable lipids, and (b) carbonate-bound lipids of the microbial mat layers (in % of the total).

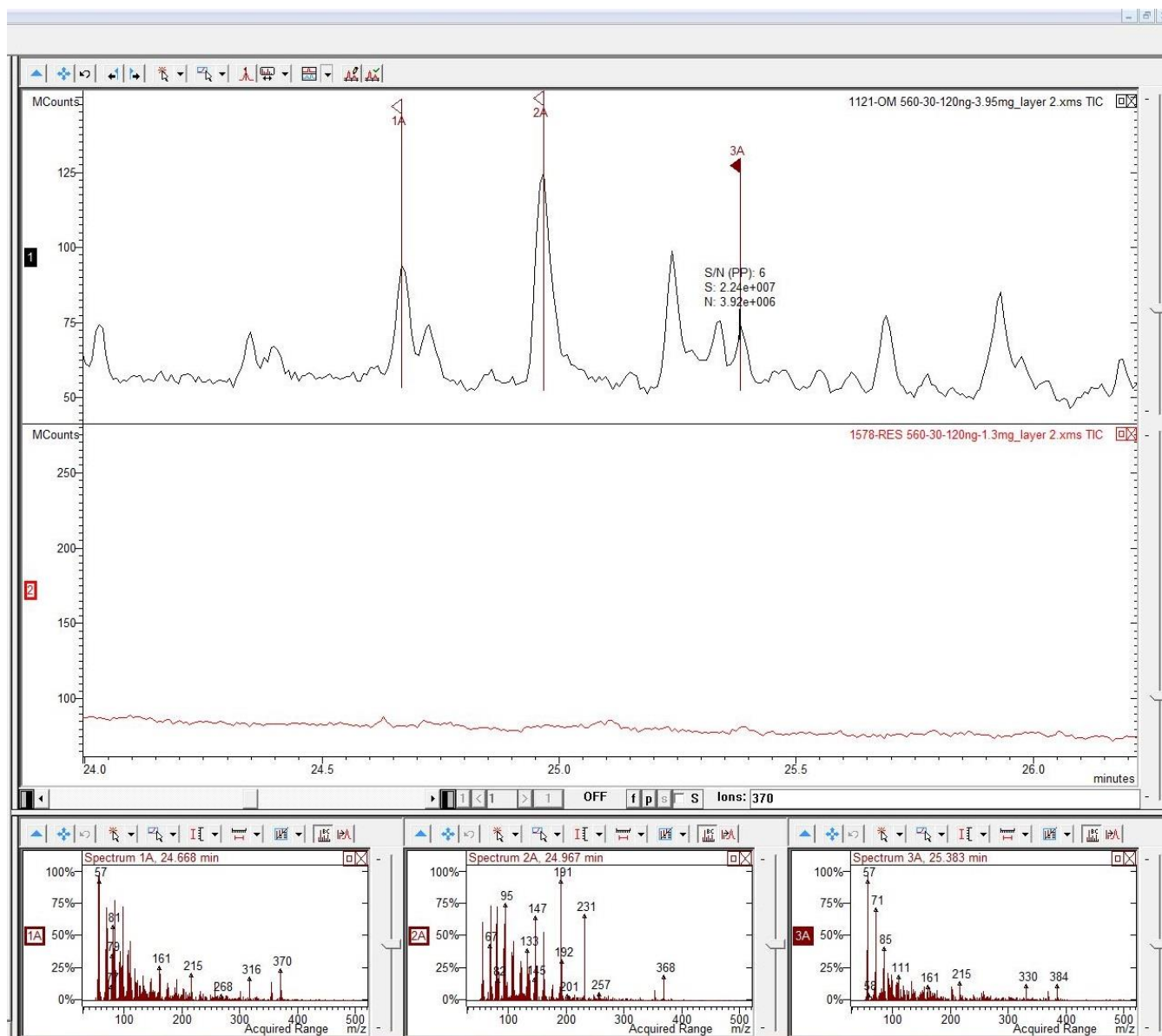


Figure S4. A screenshot showing Py-GC-MS chromatograms (TICs) of the original microbial mat (Layer 2; upper chromatogram, and its extraction residue ('kerogen'; lower chromatogram). Sterenes and hopenes are abundant in the original mat sample, but their concentrations are below detection limit in the kerogen. Mass spectra for some compounds from the upper chromatogram are also given, representing a C₂₇ sterene (1A), a C₂₇ hopene (2A), and a C₂₈ sterene (3A). Compound 3A is just identifiable from the TIC (though some coelution is evident in the mass spectrum) and it was therefore used to define our detection limit of ~1 ng (absolute amount) for kerogen-bound steroids.

Supplementary Table S1

Concentrations of major fatty acids (FAs) and hopanoids (hop-17(21)-ene, hop-22(29)-ene, $\beta\beta$ -bishomohopanoic acid, $\beta\beta$ -bishomohopan-32-ol and $\alpha\beta$ -trishomohopan-32-ol) in freely extractable lipids of the microbial mat layers ($\mu\text{g/g}$ dry mat).

Layer \ Compound	1	2	3	4	5	6
FAs	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
14:0	3.28	0.77	0.11	0.10	0.27	0.18
<i>i</i> -15:0	4.02	2.18	0.21	0.13	0.25	0.20
<i>ai</i> -15:0	0.81	1.19	0.09	0.08	0.16	0.11
15:0	2.21	1.04	0.10	0.06	0.16	0.13
<i>i</i> -16:0	7.85	1.74	0.17	0.22	0.43	0.32
16:1	26.66	0.50	--	--	--	0.89
16:0	35.85	7.17	0.89	0.94	2.56	1.97
<i>i</i> -17:0	1.18	0.59	0.08	0.10	0.18	0.16
<i>ai</i> -17:0	1.16	0.82	0.07	0.15	0.27	0.20
17:0	2.30	1.08	0.08	0.08	0.22	0.18
18:1	50.71	5.67	0.26	0.24	0.87	0.80
18:0	12.41	3.10	0.37	0.45	--	1.38
9,10-cyclopropyl-19:0	56.09	7.17	0.55	0.35	0.45	0.55
19:0	0.96	0.48	0.02	0.07	0.16	0.13
3-hydroxy-methylester (16:0)	1.90	0.20	--	0.11	0.13	0.19
20:0	1.64	0.54	0.11	0.15	0.41	0.37
21:0	0.95	0.12	--	0.03	0.09	0.09
22:0	0.66	0.34	0.03	0.14	0.45	0.35
23:0	0.17	0.09	--	0.03	0.05	0.08
24:0	1.56	0.64	0.13	0.76	3.24	2.61
26:0	0.35	0.50	0.07	0.32	0.96	0.97
28:0	--	0.52	--	0.17	0.30	0.25
30:0	--	0.28	--	0.06	0.29	0.39
Hopanoids						
Hop-17(21)-ene (Moretene)	6.67	2.82	0.38	0.48	1.84	1.05
Hop-22(29)-ene (Diploptene)	6.27	1.25	0.61	0.20	0.81	0.31
$\beta\beta$ -bishomohopanoic acid	2.17	7.74	0.79	1.78	2.60	2.59
$\beta\beta$ -bishomohopanol	1.56	3.24	1.26	0.88	3.32	1.06
$\alpha\beta$ -trishomohopanol	--	0.71	0.94	--	1.19	0.69

Supplementary Table S2

Concentrations of major fatty acids (FAs) and hopanoids (Hop-17(21)-ene, Hop-22(29)-ene, $\beta\beta$ -bishomohopanoic acid, $\beta\beta$ -bishomohopan-32-ol) in carbonate-bound lipids of the microbial mat layers ($\mu\text{g/g}$ dry mat).

Layer Compound	1	2	3	4	5	6
FAs	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$	$\mu\text{g/g}$
14:0	0.37	0.25	0.65	0.19	0.18	0.12
<i>i</i> -15:0	0.70	0.74	3.71	0.54	0.51	0.43
<i>ai</i> -15:0	0.21	0.23	0.46	0.12	0.13	0.11
15:0	0.28	0.42	0.92	0.27	0.26	0.26
<i>i</i> -16:0	0.61	0.78	3.41	0.76	0.49	0.55
16:1	1.13	0.36	--	--	--	--
16:0	5.49	2.62	12.19	2.34	1.48	1.36
<i>i</i> -17:0	0.12	0.31	1.34	0.26	0.14	0.19
<i>ai</i> -17:0	0.14	0.31	1.31	0.27	0.18	0.22
17:0	0.30	0.56	0.89	0.24	0.18	0.21
18:1	3.49	0.91	1.41	0.31	--	0.12
18:0	2.59	1.29	4.97	1.36	0.62	0.61
9,10-cyclopropyl-19:0	3.17	4.92	33.82	5.61	1.28	2.96
19:0	0.05	0.17	0.25	0.11	0.09	0.04
3-hydroxy-methylester (12:0)	--	0.10	0.23	0.28	0.05	0.04
3-hydroxy-methylester (14:0)	--	0.05	0.42	0.26	0.06	0.10
3-hydroxy-methylester (16:0)	--	0.25	3.99	1.45	0.11	0.27
20:0	0.08	0.18	0.62	0.31	0.06	0.08
21:0	0.50	0.15	1.83	0.35	0.20	0.06
22:0	0.10	0.17	1.04	0.59	0.11	0.07
24:0	0.21	0.45	2.41	2.79	0.60	0.67
26:0	0.09	0.15	0.65	0.85	0.14	0.17
28:0	--	0.12	0.49	0.47	--	--
Dioic acids-21:0	--	--	0.29	0.18	--	--
Dioic acids-22:0	--	--	0.38	0.17	--	--
Dioic acids-23:0	--	--	0.79	0.21	--	--
Dioic acids-24:0	--	--	0.27	0.18	--	--
Dioic acids-25:0	--	--	0.29	0.11	--	--
Dioic acids-26:0	--	--	0.38	0.15	--	--
Dioic acids-27:0	--	--	0.30	0.23	--	--
Dioic acids-28:0	--	--	0.74	0.26	--	--
						--
Hopanoids						--
Hop-17(21)-ene (Moretene)	--	--	1.03	0.15	--	--
Hop-22(29)-ene (Diploptene)	--	--	--	--	--	--
$\beta\beta$ -bishomohopanoic acid	--	0.72	2.19	1.76	0.22	0.22
$\beta\beta$ -bishomohopanol	--	0.22	--	0.33	--	0.50

Supplementary Table S3

Concentrations of summed steroids, major hopanoids (hop-17(21)-ene, hop-22(29)-ene, $\beta\beta$ -bishomohopanoic acid, $\beta\beta$ -bishomohopan-32-ol and $\alpha\beta$ -trishomohopan-32-ol), and fatty acids (FAs) ($\mu\text{g/g}$ dry mat) as well as steroid/hopanoid and steroid/FA ratios in freely extractable lipids of the microbial mat layers.

Layer	Steroids (C ₂₇ -C ₂₉)	Hopanoids	FAs	Steroids*/ Hopanoids	Steroids*/FAs
1	26.05	16.67	212.72	1.94	0.15
2	2.22	15.76	36.73	0.26	0.11
3	0.19	3.98	3.34	0.16	0.19
4	0.15	3.34	4.74	0.20	0.14
5	0.57	9.76	11.9	0.43	0.36
6	0.19	5.70	12.5	0.18	0.08

*including C₃₁-sterols

Supplementary Table S4

Concentrations of summed steroids, major hopanoids (hop-17(21)-ene, hop-22(29)-ene, $\beta\beta$ -bishomohopanoic acid, $\beta\beta$ -bishomohopan-32-ol), and fatty acids (FAs) ($\mu\text{g/g}$ dry mat) as well as steroid/hopanoid and steroid/FA ratios in carbonate-bound lipids of the microbial mat layers.

Layer	Steroids (C ₂₇ -C ₂₉)	Hopanoids	FAs	Steroids*/ Hopanoids	Steroids*/FAs
1	--	--	19.63	--	--
2	0.27	0.94	15.49	0.65	0.04
3	0.32	3.22	80.45	0.22	0.01
4	0.79	2.24	21.22	0.55	0.06
5	0.10	0.22	6.87	1.44	0.05
6	0.14	0.72	8.64	1.01	0.08

*including C₃₁-sterols