Supplementary material for:

Bacteriohopanepolyols track environmental transitions in the Black Sea

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Content:

S1. BHP structures and identification

S1.1 BHP structures

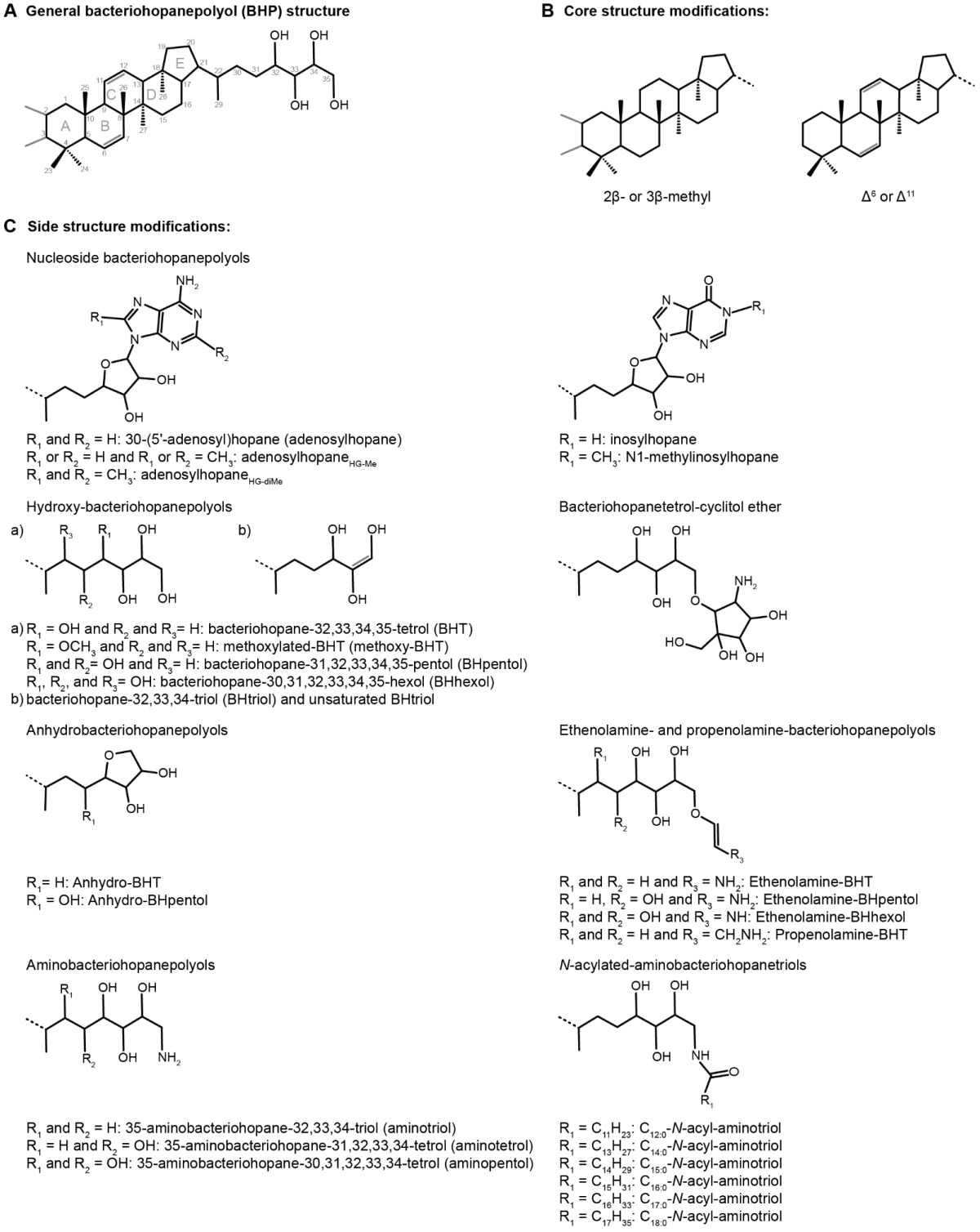
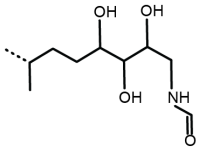
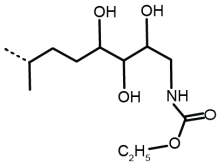


Figure S1. Structures of bacteriohopanepolyols (BHPs) identified in this study. **(A)** A general BHP structure with the core hopane and extended side chain (example of BHT). **(B)** Common modifications to the core BHP structure, including additional methyl groups and unsaturations on the core structure. **(C)** Side chain configurations discussed in this study. Note: for methoxylated-BHT and unsaturated BHtriol, the placement of the methoxy group and unsaturation, respectively, are arbitrary.

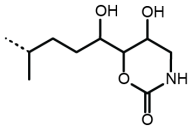
N-formylated-35-aminobacteriohopanetriol



Ethylcarbamate-aminobacteriohopanetriol



Oxazinone-aminobacteriohopanetriol



Dioxanone-methylaminobacteriohopanetriol

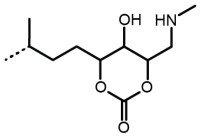


Figure S2. BHP side chain configurations continued.

S1.2 List of BHPs

Table S1. List of BHPs identified in this study with the retention time (tr), assigned elemental composition (AEC), calculated exact mass (M_{calc}), and Δppm (where Δppm = $((M_{calc}-M_{measured})/(M_{calc}) \times 10^6$). Note: methyl group positions for nucleoside BHPs (adenosylhopanes and inosylhopanes) were determined based on retention times. BHP unsaturation positions were determined either based on the MS^2 or on the difference in retention time between the unsaturated BHP and its saturated counterpart.

BHP	Depth (cm)	tr (min)	AEC	MS^2 Ion	M_{calc} (*m/z*)	Δ ppm
Adenosylhopane	220	21.77	$C_{40}H_{64}O_3N_5$	H^+	662.500	-0.61
2Me-adenosylhopane	220	21.88	$C_{41}H_{66}O_3N_5$	H^+	676.516	-0.18
adenosylhopaneHG-Me	220	22.93	$C_{41}H_{66}O_3N_5$	H^+	676.516	-0.41
3Me-adenosylhopane	220	23.66	$C_{41}H_{66}O_3N_5$	H^+	676.516	0.37
2Me-adenosylhopaneHG-Me	220	22.89	$C_{42}H_{68}O_3N_5$	H^+	690.532	-0.85
Me-adenosylhopaneHG-Me	220	23.60	$C_{42}H_{68}O_3N_5$	H^+	690.532	-0.43
adenosylhopaneHG-diMe	220	25.09	$C_{42}H_{68}O_3N_5$	H^+	690.532	-0.52
Me-adenosylhopaneHG-diMe	220	17.82	$C_{43}H_{70}O_3N_5$	H^+	704.547	-0.54
diMe-adenosylhopaneHG-Me	220	23.62	$C_{43}H_{70}O_3N_5$	H^+	704.547	-0.60
2Me-adenosylhopaneHG-diMe	220	25.17	$C_{43}H_{70}O_3N_5$	H^+	704.547	0.03
Me-adenosylhopaneHG-diMe	220	26.09	$C_{43}H_{70}O_3N_5$	H^+	704.547	-0.13
3Me-adenosylhopaneHG-diMe	220	27.54	$C_{43}H_{70}O_3N_5$	H^+	704.547	-0.07
2,3diMe-adenosylhopaneHG-diMe	220	26.17	$C_{44}H_{72}O_3N_5$	H^+	718.563	0.25
inosylhopane	220	20.23	$C_{40}H_{63}O_4N_4$	H^+	663.484	-0.06
N1-methylinosylhopane	220	20.33	$C_{41}H_{65}O_4N_4$	H^+	677.500	-0.93
N1-methylinosylhopane	220	23.90	$C_{41}H_{65}O_4N_4$	H^+	677.500	-0.53
2Me-N1-methylinosylhopane	220	20.55	$C_{42}H_{67}O_4N_4$	H^+	691.516	-0.10
Me-N1-methylinosylhopane	220	22.11	$C_{42}H_{67}O_4N_4$	H^+	691.516	-0.14
Me-N1-methylinosylhopane	220	23.96	$C_{42}H_{67}O_4N_4$	H^+	691.516	0.91
BHtriol	130	21.30	$C_{34}H_{64}O_3N$	NH_4^+	534.489	0.80
Unsat. BHtriol	130	23.26	$C_{34}H_{62}O_3N$	NH_4^+	532.472	-0.56
BHT (22*R*, 34*S*)	130	20.48	$C_{35}H_{66}O_4N$	NH_4^+	564.499	-1.09
BHT-*x*	130	21.01	$C_{35}H_{66}O_4N$	NH_4^+	564.499	-1.42
2Me-BHT	130	20.69	$C_{36}H_{68}O_4N$	NH_4^+	578.514	-1.64
Methoxylated-BHT	130	22.50	$C_{36}H_{68}O_4N$	NH_4^+	578.514	-1.76
Methoxylated-BHT	95	22.89	$C_{36}H_{68}O_4N$	NH_4^+	578.514	-1.26
BHT-CE	130	17.04	$C_{41}H_{74}O_8N$	H^+	708.541	-1.22
Anhydro-BHT	220	24.31	$C_{35}H_{64}O_3N$	NH_4^+	546.488	-0.09
BHpentol	220	18.53	$C_{35}H_{66}O_5N$	NH_4^+	580.494	-0.47
Anhydro-BHpentol	130	18.22	$C_{35}H_{64}O_4N$	NH_4^+	562.483	-1.40
Anhydro-BHpentol	130	19.86	$C_{35}H_{64}O_4N$	NH_4^+	562.483	-1.39
Anhydro-BHpentol	130	21.77	$C_{35}H_{64}O_4N$	NH_4^+	562.483	-1.48
BHhexol	220	16.37	$C_{35}H_{63}O_6$	H^+	579.462	0.26
Propenolamine-BHT	130	21.17	$C_{38}H_{68}O_4N$	H^+	602.514	-1.18
Unsat. propenolamine-BHT	95	18.70	$C_{38}H_{66}O_4N$	H^+	600.498	-0.77

Ethenolamine-BHT	130	20.66	$C_{37}H_{66}O_4N$	H^+	588.499	-0.70
Unsat. Ethenolamine-BHT	130	17.54	$C_{37}H_{64}O_4N$	H^+	586.483	-0.81
Unsat. Ethenolamine-BHT	130	18.16	$C_{37}H_{64}O_4N$	H^+	586.483	-0.83
Methoxylated-ethenolamine-BHT	95	22.55	$C_{38}H_{68}O_4N$	H^+	602.515	-0.13
Ethenolamine-BHpentol	130	18.69	$C_{37}H_{66}O_5N$	H^+	604.494	-1.69
Ethenolamine-BHhexol	95	16.52	$C_{37}H_{66}O_6N$	H^+	620.488	-1.00
Aminotriol isomer	95	16.85	$C_{35}H_{64}O_3N$	H^+	546.488	-1.83
Aminotriol	95	17.44	$C_{35}H_{64}O_3N$	H^+	546.488	-0.33
Δ^6-aminotriol	130	15.30	$C_{35}H_{62}O_3N$	H^+	544.472	-0.77
Δ^{11}-aminotriol	130	15.66	$C_{35}H_{62}O_3N$	H^+	544.472	-1.19
$C_{12:0}$-N-acyl-aminotriol	130	30.26	$C_{47}H_{86}O_4N$	H^+	728.655	-1.07
$C_{12:0}$-N-acyl-aminotriol	130	30.88	$C_{47}H_{86}O_4N$	H^+	728.655	-1.62
$C_{14:0}$-N-acyl-aminotriol	130	33.46	$C_{49}H_{90}O_4N$	H^+	756.686	-0.44
$C_{14:0}$-N-acyl-aminotriol	130	34.39	$C_{49}H_{90}O_4N$	H^+	756.686	-1.18
$C_{15:0}$-N-acyl-aminotriol	130	35.27	$C_{50}H_{92}O_4N$	H^+	770.702	-0.17
$C_{15:0}$-N-acyl-aminotriol	130	36.20	$C_{50}H_{92}O_4N$	H^+	770.702	0.34
$C_{16:0}$-N-acyl-aminotriol	130	37.11	$C_{51}H_{94}O_4N$	H^+	784.718	-0.55
$C_{16:0}$-N-acyl-aminotriol	130	37.99	$C_{51}H_{94}O_4N$	H^+	784.718	0.22
$C_{17:0}$-N-acyl-aminotriol	95	38.41	$C_{52}H_{96}O_4N$	H^+	798.733	-1.50
$C_{17:0}$-N-acyl-aminotriol	130	38.81	$C_{52}H_{96}O_4N$	H^+	798.733	-2.10
$C_{17:0}$-N-acyl-aminotriol	95	39.81	$C_{52}H_{96}O_4N$	H^+	798.733	-0.11
$C_{18:0}$-N-acyl-aminotriol	130	40.53	$C_{53}H_{98}O_4N$	H^+	812.749	0.10
$C_{18:0}$-N-acyl-aminotriol	130	41.43	$C_{53}H_{98}O_4N$	H^+	812.749	-1.70
Aminotetrol	220	16.17	$C_{35}H_{64}O_4N$	H^+	562.483	-1.74
Aminopentol	220	14.56	$C_{35}H_{64}O_5N$	H^+	578.478	-1.04
N-formylated-aminotriol	220	20.37	$C_{36}H_{64}O_4N$	H^+	574.483	-0.96
Δ^6-N-formylated aminotriol	130	18.01	$C_{36}H_{62}O_4N$	H^+	572.467	-1.72
Ethylcarabamte-aminotriol	130	18.44	$C_{38}H_{68}O_5N$	H^+	618.509	-1.02
Ethylcarabamte-aminotriol	130	19.48	$C_{38}H_{68}O_5N$	H^+	618.509	-1.29
Dioxanone-methylaminotriol	130	21.57	$C_{37}H_{64}O_4N$	H^+	586.483	-1.34
Oxazinone-aminotriol	130	20.75	$C_{36}H_{62}O_4N$	H^+	572.467	-0.41

S1.3 Identification of methoxylated-ethenolamine-BHT

A search for propenolamine-BHT in the partial mass chromatogram of m/z 602.514 revealed an additional peak at 22.54 mins. In the MS^2 spectrum of m/z 602.514 (AEC $C_{38}H_{68}O_4N^+$, Δppm -0.13) we observe the loss of one hydroxyl group that yields m/z 584.501 ($C_{38}H_{66}O_3N^+$, Δppm 1.15) followed by a loss of 32 Da (m/z 552.476, $C_{37}H_{62}O_2N^+$, Δppm 1.13) and another hydroxyl group (m/z 534.467, $C_{37}H_{60}ON^+$ Δppm 0.77). This suggests that the compound contains at least two hydroxyl groups and a methoxylated group on the side chain. The additional loss of 59 Da (m/z 475.429, $C_{35}H_{55}^+$, Δppm 1.55) suggests that the side chain contains an ethenolamine group. Therefore, we tentatively identify this compound as a methoxylated-ethenolamine-BHT (methoxy-ethenolamine-BHT), where the methoxylated group could replace any of the three hydroxyl groups in the ethenolamine-BHT structure.

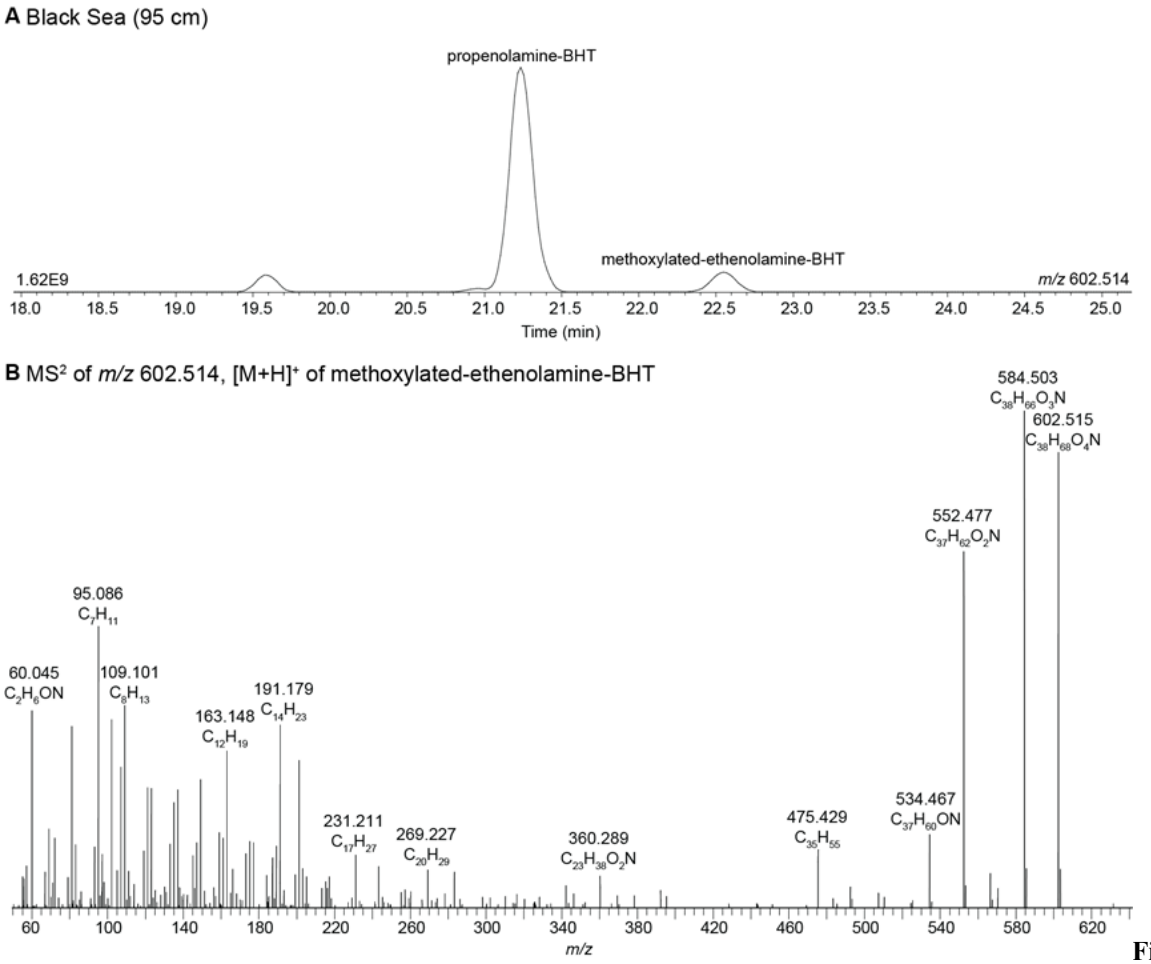


Figure S3. (A) Partial mass chromatogram of propenolamine-BHT and methoxylated-ethenolamine-BHT from a sample in the Black Sea at 95 cm depth with the exact mass and intensity of the highest peak in arbitrary units (AU). **(B)** MS^2 of the novel methoxylated-ethenolamine-BHT.

S2. BHP distributions downcore

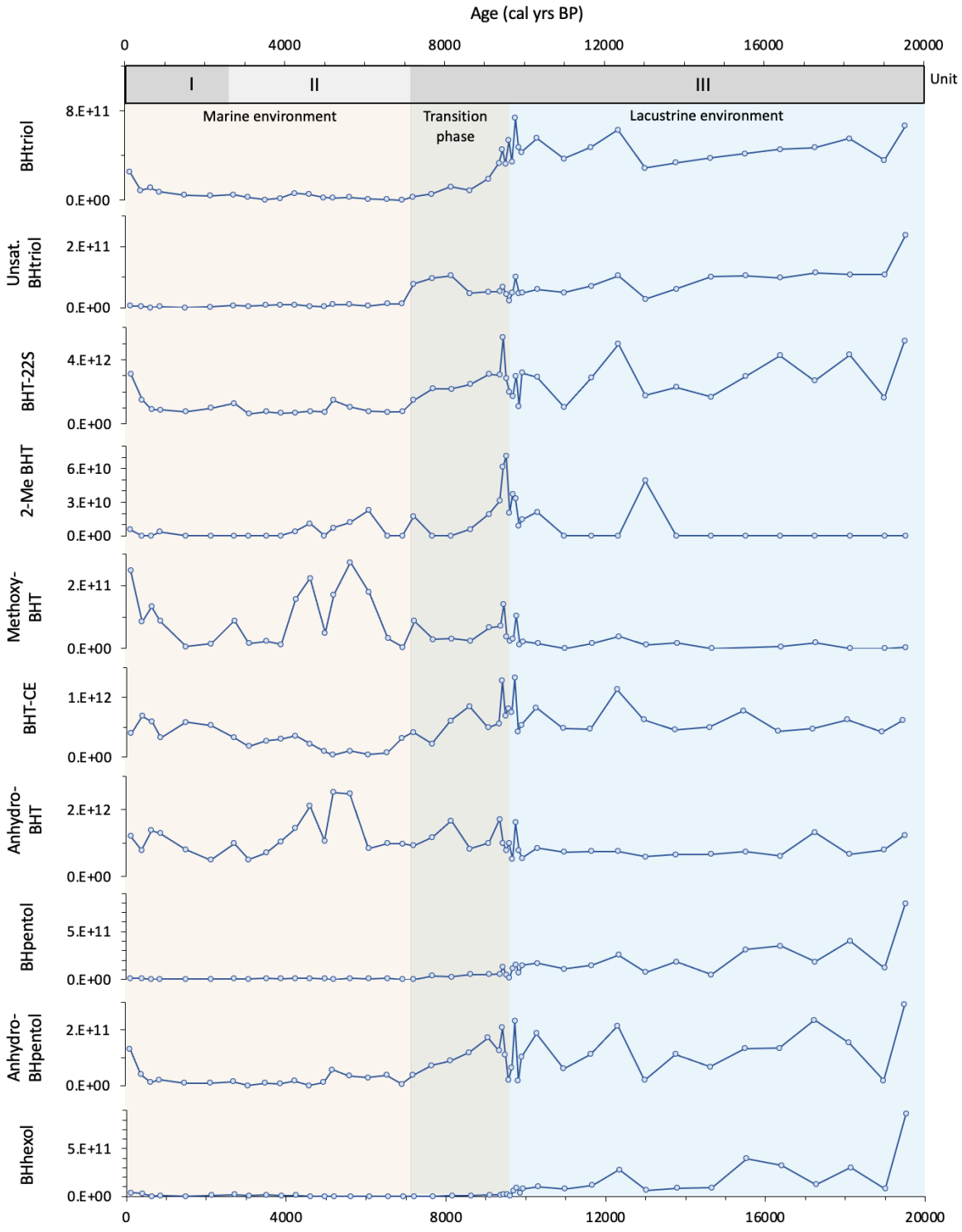


Figure S4: Changes over the last 19.5 ka in the absolute abundance (peak area per g TOC) of BHPs identified in core 64PE418

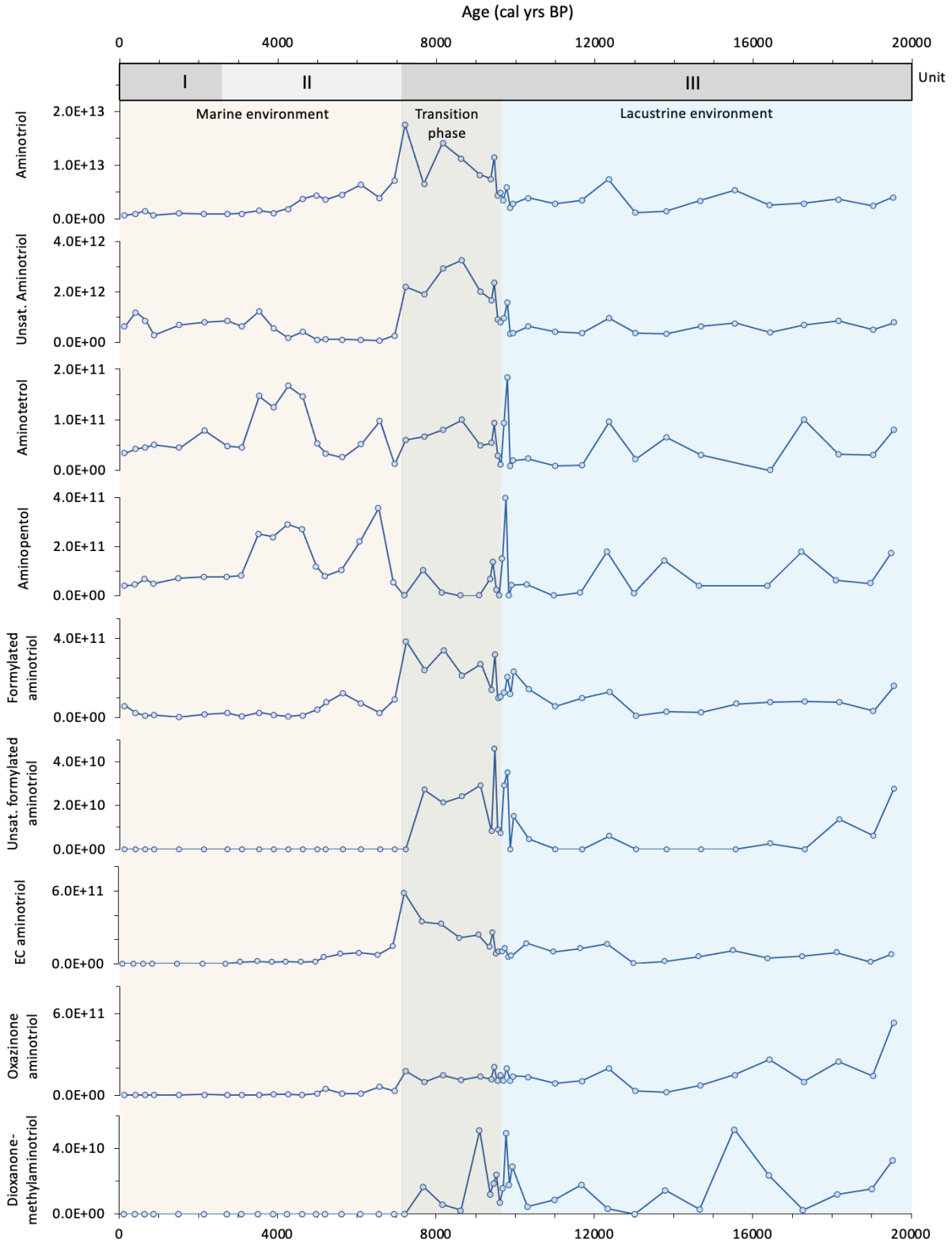


Figure S5: Changes over the last 19.5 ka in the absolute abundance (peak area per g TOC) of BHPs identified in core 64PE418

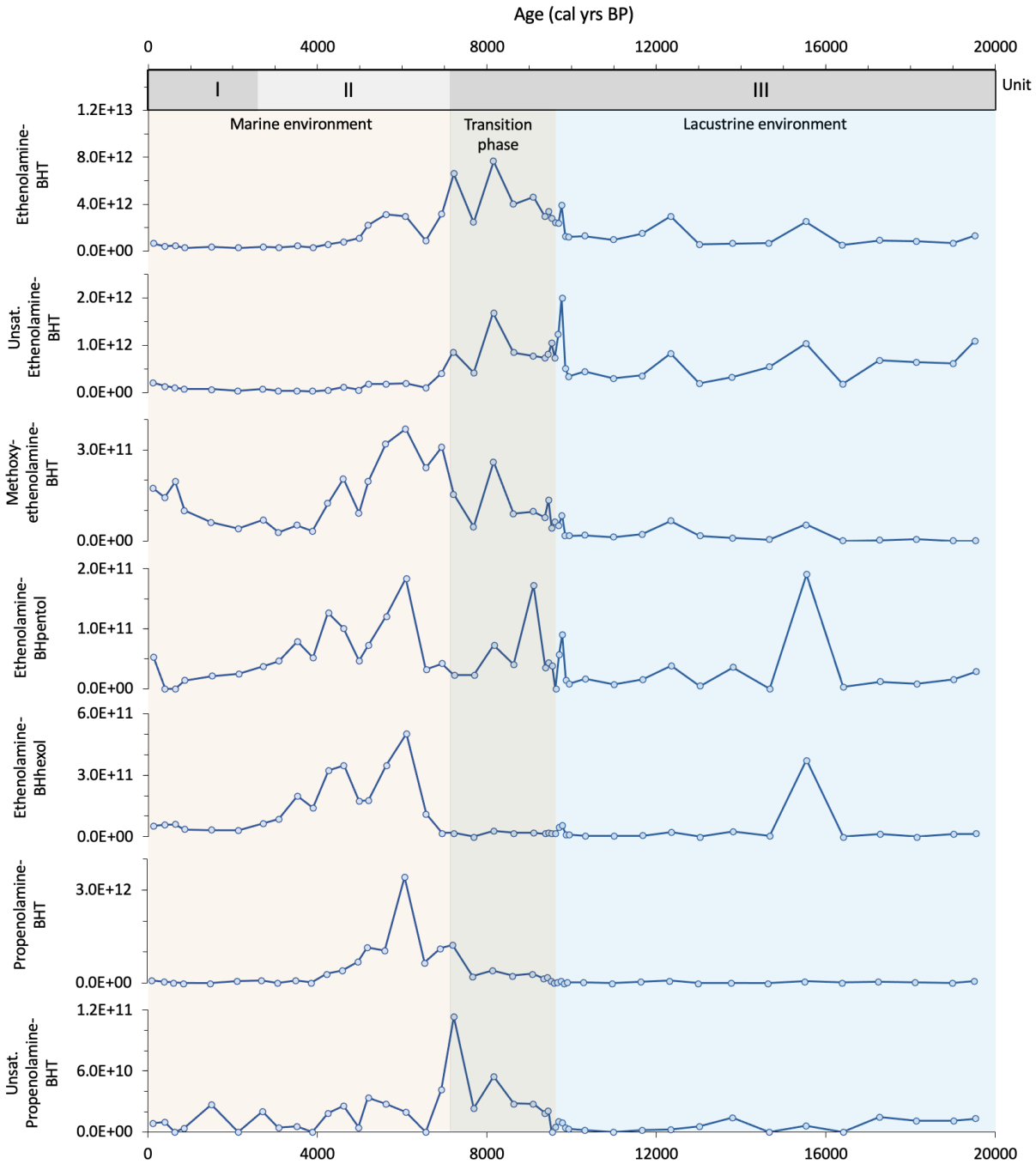


Figure S6: Changes over the last 19.5 ka in the absolute abundance (peak area per g TOC) of BHPs identified in core 64PE418

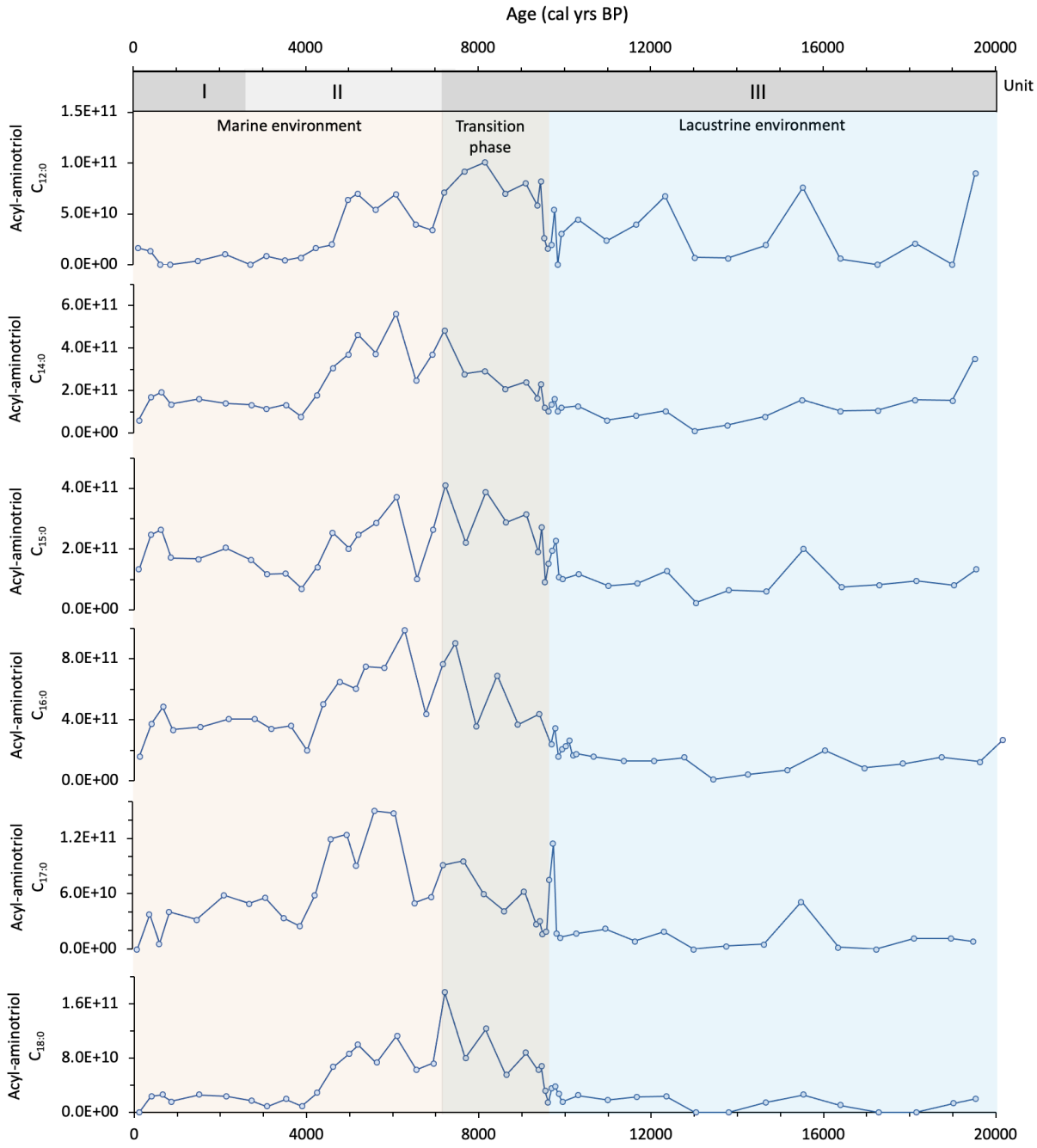


Figure S7: Changes over the last 19.5 ka in the absolute abundance (peak area per g TOC) of Acyl-aminotriols identified in core 64PE418

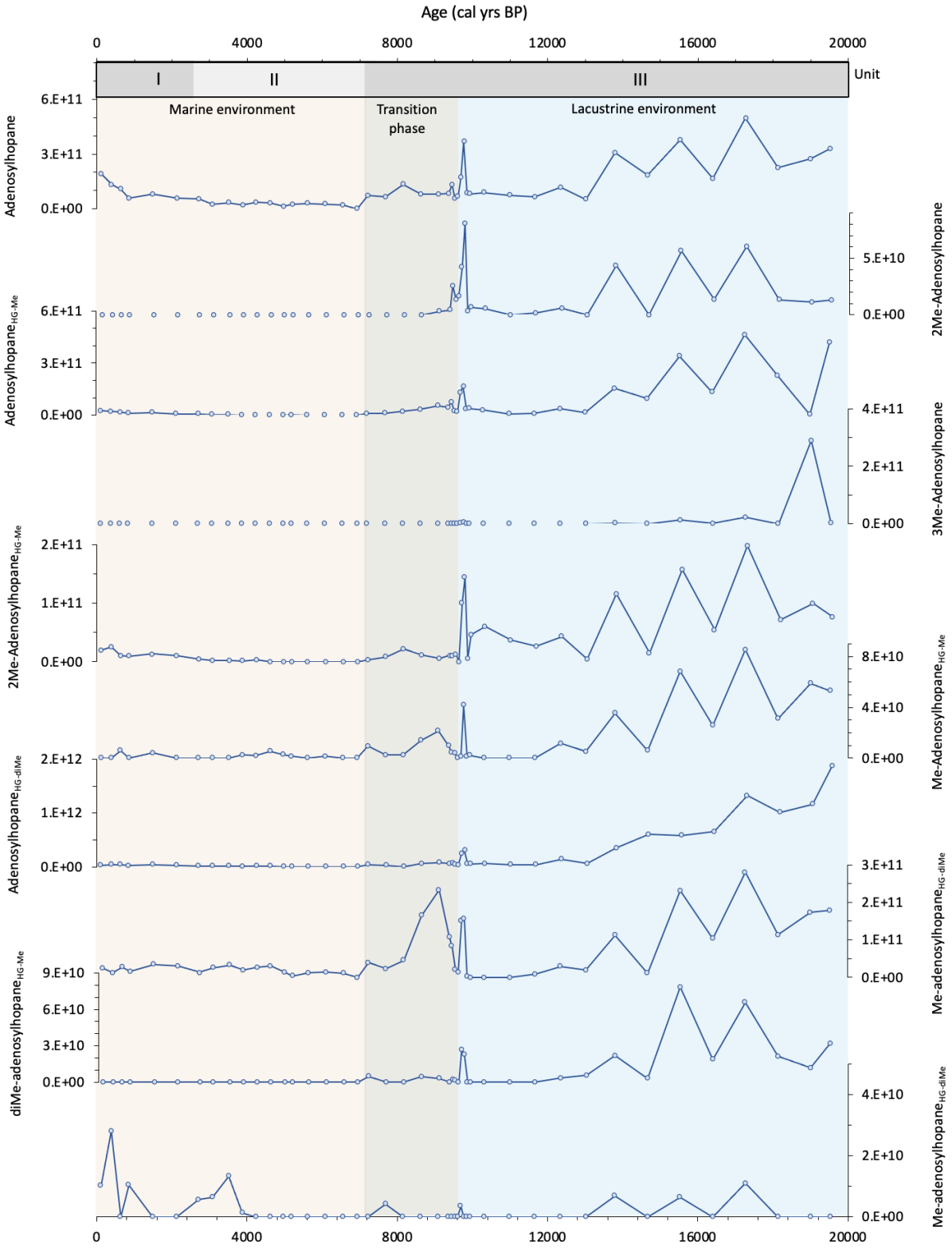


Figure S8: Changes over the last 19.5 ka in the absolute abundance (peak area per g TOC) of all Nu-BHPs identified in core 64PE418

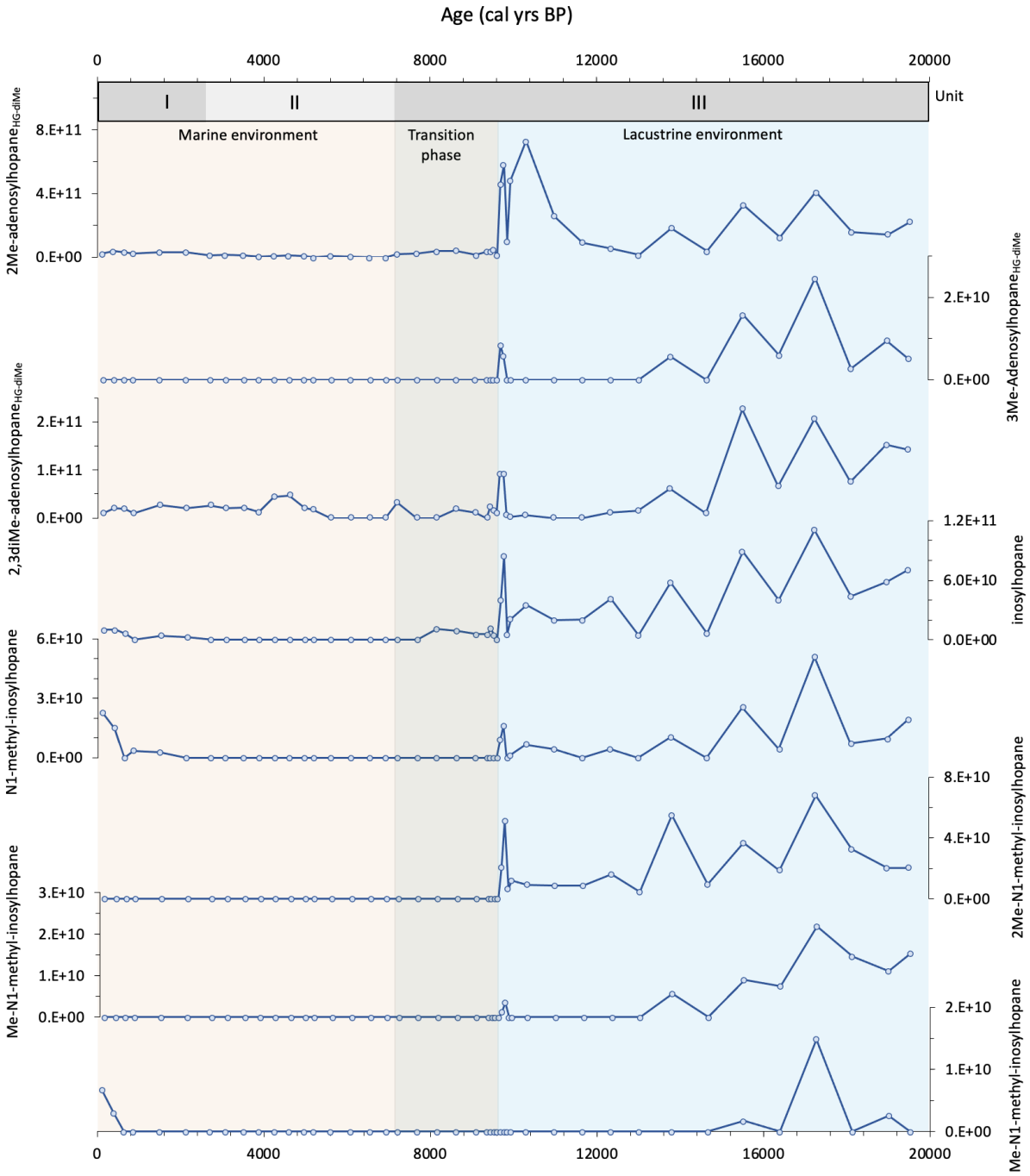


Figure S9: Changes over the last 19.5 ka in the absolute abundance (peak area per g TOC) of all Nu-BHPs identified in core 64PE418

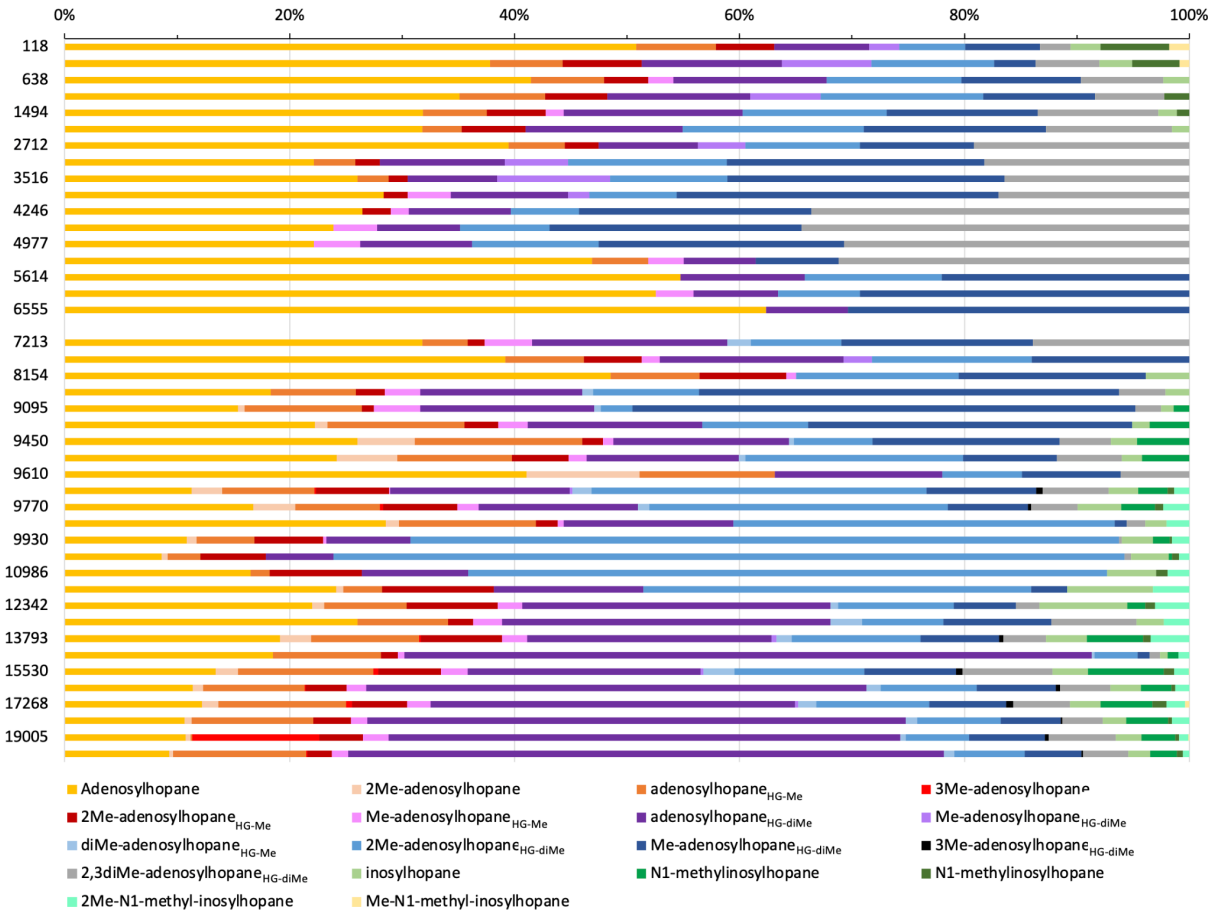


Figure S10: Changes over the last 19.5 ka in the relative abundance of Nu-BHPs identified in core 64PE418