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Occurrence and abundance of soil-specific bacterial membrane lipid markers in the Têt watershed (southern France): Soil-specific BHPs and branched GDGTs

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[1] Recently, four bacteriohopanepolyols (BHPs), adenosylhopane, and structurally similar adenosylhopane-type 1, 2-methyl adenosylhopane, and 2-methyl adenosylhopane-type 1, have been suggested to be characteristic of soil microbial communities and therefore can serve as molecular markers for soil organic matter (OM) supply in river, lake, and marine sediments. In this study, we analyzed BHPs in peats and soils collected in the Têt watershed (southern France) and compared them with branched glycerol dialkyl glycerol tetraethers (GDGTs), a more established molecular tracer of soil OM. Adenosylhopane-type I is identified in all of the samples from the study area except one collected near the Têt River mouth with up to three of the related compounds also frequently present, particularly in the surface samples. The concentrations of soil-specific BHPs in peat environments have been shown to increase with lower $\delta^{15}N$ values, providing evidence that N_2-fixing bacteria are probably a major source of soil-specific BHPs in acidic environments. It seems likely that soil pH is a major factor controlling BHP occurrence based on statistical analysis of environmental parameters and BHP concentration data. The comparison of the soil-specific BHP concentrations with those of branched GDGTs shows no clear relationship in the Têt River system, supporting the concept that these two groups of soil-specific compounds are synthesized by different microbial organisms living in different niches in the soil profile (e.g., oxic top versus anoxic deep).

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1. Introduction

[2] Bacteriohopanepolyols (BHPs) are pentacyclic triterpenoids [*Rohmer*, 1993] biosynthesized as membrane lipids by a wide range of bacteria including cyanobacteria, nitrogen-fixing bacteria, purple nonsulfur bacteria, acetic acid bacteria, methanotrophs, and methylotrophs [e.g., *Talbot et al.*, 2008, and references therein]. Analysis of BHPs in terrestrial samples from various locations around the world showed that four BHPs, adenosylhopane (Ic; see Appendix A), 2-methyladenosylhopane (IIc), and the two related structures with an as yet undetermined terminal group structure termed "adenosylhopane-type 1" (Id) and "2-methyladenosylhopane-type 1" (IId), are common components of soils and peats [*Talbot and Farrimond*, 2007; *Cooke et al.*, 2008a; *Redshaw et al.*, 2008; *Xu et al.*, 2009; *Rethemeyer et al.*, 2010] but have rarely been observed in lacustrine [*Talbot and Farrimond*, 2007] and offshore [*Cooke et al.*, 2007] sediments. Another pair of related homologues, with a second, as yet unidentified terminal group, have recently been reported in soils from Svalbard [*Rethemeyer et al.*, 2010]. However, their occurrence seems to be more restricted than those of adenosylhopanes and adenosylhopane-type 1 compounds. Data from surface sediments from the continental shelf off the Rhône River [*Cooke et al.*, 2007] showed a steady decrease in the abundance of soil-specific BHPs with distance from the river mouth. Recent studies of Congo fan sediments [*Cooke et al.*, 2008b; *Handley et al.*, 2010] have shown evidence of the preservation of these compounds in sediments associated with transport of soil OM from the Congo up to ~100 mbsf and ~1.2 Ma. These promising initial results indicate the potential of soil-specific BHPs to serve as an indicator for soil OM input from land to the ocean. However, the use of the new soil-specific BHPs has not been widely tested yet as a robust proxy for soil OM input in various environmental settings.

[3] Here, we investigated soils collected in the Têt watershed (southern France) and determined variations in BHP concentrations. For the first time, soil BHP distributions are directly compared to environmental variables including pH, precipitation, and mean annual air temperature (MAT) to determine potential controls on BHP distributions over a range of conditions occurring within a single catchment. BHPs are also compared with a well established proxy of river-borne soil OM input to the ocean, the branched and isoprenoid tetraether (BIT) index [*Hopmans et al.*, 2004; *Huguet et al.*, 2007; *Kim et al.*, 2009]. The BIT index is based on a group of branched glycerol dialkyl glycerol tetraethers (GDGTs) derived from presumably anaerobic bacteria [*Weijers et al.*, 2006a], which occur widely in soils [*Weijers et al.*, 2006b, 2007], and a structurally related isoprenoid GDGT 'crenarchaeol', predominantly found in marine planktonic Crenarchaeota [*Sinninghe Damsté et al.*, 2002].

2. Study Area

[4] The Têt watershed, a typical Mediterranean river system, is located in the southern part of France (Figure 1). The uppermost part of the Têt watershed is situated in elevated regions with steep slopes [*Garcia-Esteves et al.*, 2007]. In general, soils are thin in the upstream area of the Têt River. They are mostly cambisols with the vegetation essentially composed of pasture grass. Peats also occur in some places in the upper Têt watershed. The surrounding forest is mainly composed of beech as well as Douglas and Laricio pine trees. Further downstream, intensive agricultural land use

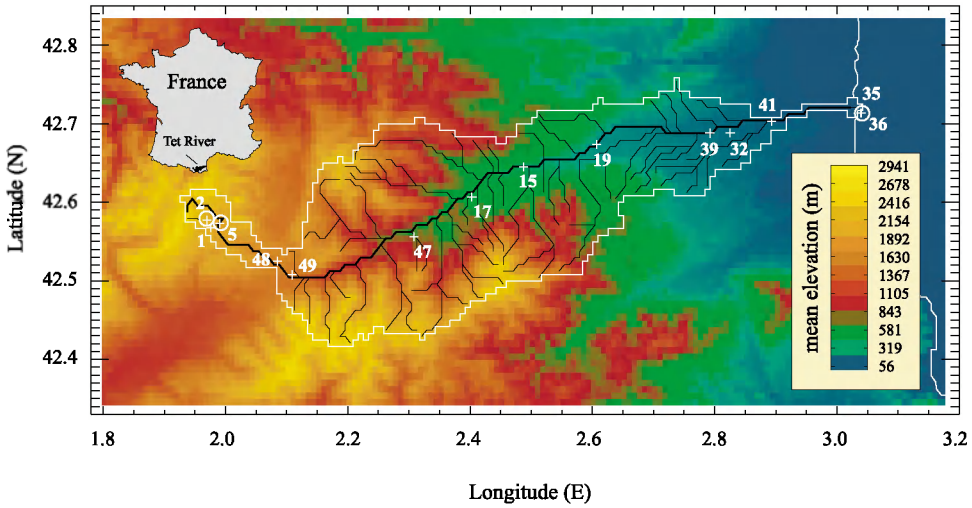

Figure 1. Map showing soil sampling sites in the Têt watershed. Open circles indicate the sites where soil depth profiles were studied. Numbers correspond to sample code TESO numbers in Table 1.

becomes dominant in the form of orchards and vineyards.

3. Material and Methods

3.1. Sample Collection and Preparation

[5] Sampling of Têt soils (TESO) was carried out in June and July 2007 along the Têt River, from the source area in the Pyrenees to the river mouth into the Gulf of Lions (Figure 1 and Table 1). In total, 29 samples including 2 peats (TESO2 and TESO 49) were collected from 15 sampling sites, including 14 topsoils (i.e., upper 10 cm soils) and 3 soil profiles (TESO2, TESO5, and TESO36). All samples were immediately frozen with dry ice in the field and subsequently stored at −40°C. The soil samples were freeze-dried, sieved (<2 mm), and ground with a swing mill to obtain homogeneous material for further geochemical analysis.

3.2. Bulk Parameter Analyses

[6] Total nitrogen content (TN, Table 1) was analyzed with a Thermo Elemental Analyzer Flash EA 1112 at Royal Netherlands Institute for Sea Research (NIOZ) at least in duplicate. The analytical error is on average 0.01% for TN contents. For total nitrogen stable isotope composition ($\delta^{15}N$, Table 1), bulk soil samples were analyzed using a Flash EA 1112 Elemental Analyzer interfaced with a ThermoFinnigan Delta^{Plus} mass spectrometer at NIOZ. All samples were analyzed in triplicate. The analytical error is less than ±0.5 ‰.

3.3. BHP Analysis

[7] All the soil samples investigated were extracted at NIOZ. The extraction method is similar to that reported by *Talbot et al.* [2008], which is based on the Kates modification of the original Bligh and Dyer extraction [*Bligh and Dyer*, 1959], except for the use of dichloromethane (DCM) instead of chloroform. An aliquot of the total lipid extract was derivatised by heating with acetic anhydride and pyridine (4 ml; 1:1 v/v] at 50°C for 1 h and leaving at room temperature overnight. The derivatised extract was rotary evaporated to near dryness, transferred to a vial using DCM, blown down to dryness under N_2, and redissolved in methanol/propan-2-ol (60:40, v/v] for liquid chromatography tandem mass spectrometry (LC-MS^n) analysis. Analysis was performed as described previously [*Cooke et al.*, 2008a] using a high-performance liquid chromatography (HPLC) and detection via a Thermo Finnigan LCQ ion trap mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) source operated in positive ion mode. Structures were assigned from comparison with published spectra where possible [*Talbot et al.*, 2003a, 2003b, 2007a, 2007b, 2008]. A semiquantitative estimate of BHP abundance was calculated from the characteristic base peak areas of individual BHPs in mass chromatograms relative to the m/z 345 ([M + H-CH_3COOH]$^+$) base peak area

Table 1. Sampling Sites, Environmental Data, and Results of Bulk Geochemical, Branched GDGT, and BHP Analyses From the Investigated Têt Soil Samples[a]

Sample Code	Material	Sampling Depth (cm)	Longitude (E)	Latitude (N)	Altitude (m)	Sampling Date dd/mm/yyyy	MAT[b] (°C)	Precipitation[b] (mm)	Soil pH[b]	TN (wt. %)	$\delta^{15}N$ (‰)	Branched GDGT[b] ($\mu g\ g_{TOC}^{-1}$)	BIT Index[b]	Total BHPs ($\mu g\ g_{TOC}^{-1}$)	Soil-Specific BHPs ($\mu g\ g_{TOC}^{-1}$)	Soil-Specific BHPs (%)	BHPs[c]
TESO1	Soil	2–7	1.976	42.559	2128	11/06/2007	4	1009	4.9	0.9	2.3	25	1.0	264	130	49	13
TESO2	Peat	0–10	1.975	42.568	2136	11/06/2007	3.9	1053	4.7	1.7	0.7	126	1.0	908	218	24	16
TESO2	Peat	10–20	1.975	42.568	2136	11/06/2007	3.9	1053	4.7	0.6	1.1	141	1.0	2289	470	21	11
TESO2	Peat	37	1.975	42.568	2136	11/06/2007	3.9	1053	5.5	0.0	n.d	154	1.0	516	150	29	5
TESO5	Soil	2–7	2.000	42.576	2157	11/06/2007	3.7	1098	5.2	0.3	7.6	17	1.0	138	69	50	10
TESO5	Soil	7–12	2.000	42.576	2157	11/06/2007	3.7	1098	5.0	0.6	5.0	22	1.0	622	325	52	15
TESO5	Soil	12–17	2.000	42.576	2157	11/06/2007	3.7	1098	5.0	0.4	5.7	34	1.0	275	139	51	10
TESO5	Soil	17–22	2.000	42.576	2157	11/06/2007	3.7	1098	5.1	0.4	6.1	29	1.0	285	122	43	12
TESO5	Soil	22–27	2.000	42.576	2157	11/06/2007	3.7	1098	5.2	0.4	6.4	17	1.0	256	92	36	9
TESO5	Soil	27–32	2.000	42.576	2157	11/06/2007	3.7	1098	5.0	0.4	6.7	21	1.0	191	86	45	10
TESO5	Soil	32–37	2.000	42.576	2157	11/06/2007	3.7	1098	5.1	0.4	6.8	14	1.0	291	98	34	9
TESO15	Soil	1–5	2.487	42.645	271	12/06/2007	13.8	624	7.2	0.1	2.6	10	1.0	358	132	37	8
TESO17	Soil	upper 10	2.402	42.607	437	12/06/2007	13.8	599	8.4	0.4	6.3	2	0.3	42	20	47	4
TESO19	Soil	upper 10	2.606	42.674	151	12/06/2007	14.2	633	7.9	0.1	1.5	4	0.7	408	266	65	8
TESO32	Soil	upper 10	2.792	42.688	60	13/06/2007	15.3	634	6.8	0.3	6.3	17	1.0	365	57	16	7
TESO35	Soil	upper 10	3.039	42.714	0	14/06/2007	15.4	577	8.9	0.0	6.7	5	0.6	353	0	0	3
TESO36	Soil	0–5	3.038	42.714	0	14/06/2007	15.4	577	7.5	0.2	5.9	12	0.6	157	31	20	6
TESO36	Soil	5–10	3.038	42.714	0	14/06/2007	15.4	577	7.8	0.2	5.9	11	0.6	191	32	17	4
TESO36	Soil	10–15	3.038	42.714	0	14/06/2007	15.4	577	7.9	0.1	6.2	12	0.6	146	33	23	3
TESO36	Soil	15–20	3.038	42.714	0	14/06/2007	15.4	577	8.0	0.1	6.4	8	0.5	101	14	14	3
TESO36	Soil	20–25	3.038	42.714	0	14/06/2007	15.4	577	8.0	0.1	6.4	7	0.5	131	20	15	3
TESO36	Soil	25–30	3.038	42.714	0	14/06/2007	15.4	577	8.1	0.1	6.3	7	0.5	133	18	14	3
TESO36	Soil	30–35	3.038	42.714	0	14/06/2007	15.4	577	8.0	0.1	5.9	10	0.6	121	15	12	7
TESO36	Soil	35–40	3.038	42.714	0	14/06/2007	15.4	577	7.7	0.1	5.6	11	0.7	113	20	18	4
TESO39	Soil	upper 10	2.774	42.706	74	14/06/2007	15.2	619	7.5	0.1	5.9	3	0.6	119	63	53	9
TESO41	Soil	upper 10	2.892	42.703	25	14/06/2007	15.3	555	7.6	0.1	4.8	3	0.8	332	194	58	8
TESO47	Soil	upper 10	2.307	42.557	831	15/06/2007	10.5	549	7.0	0.4	1.2	0	0.9	255	168	66	9
TESO48	Soil	0–20	2.084	42.525	1720	13/07/2007	6.4	728	4.6	0.2	4.7	18	1.0	605	197	33	16
TESO49	Peat	0–10	2.109	42.508	1520	13/07/2007	7.7	790	4.8	1.5	2.3	101	1.0	1927	589	31	11

[a]MAT indicates mean annual air temperature. Topsoil indicates the upper 10 cm.
[b]Data from *Kim et al.* [2010].
[c]Number of BHP structures identified in each sample.

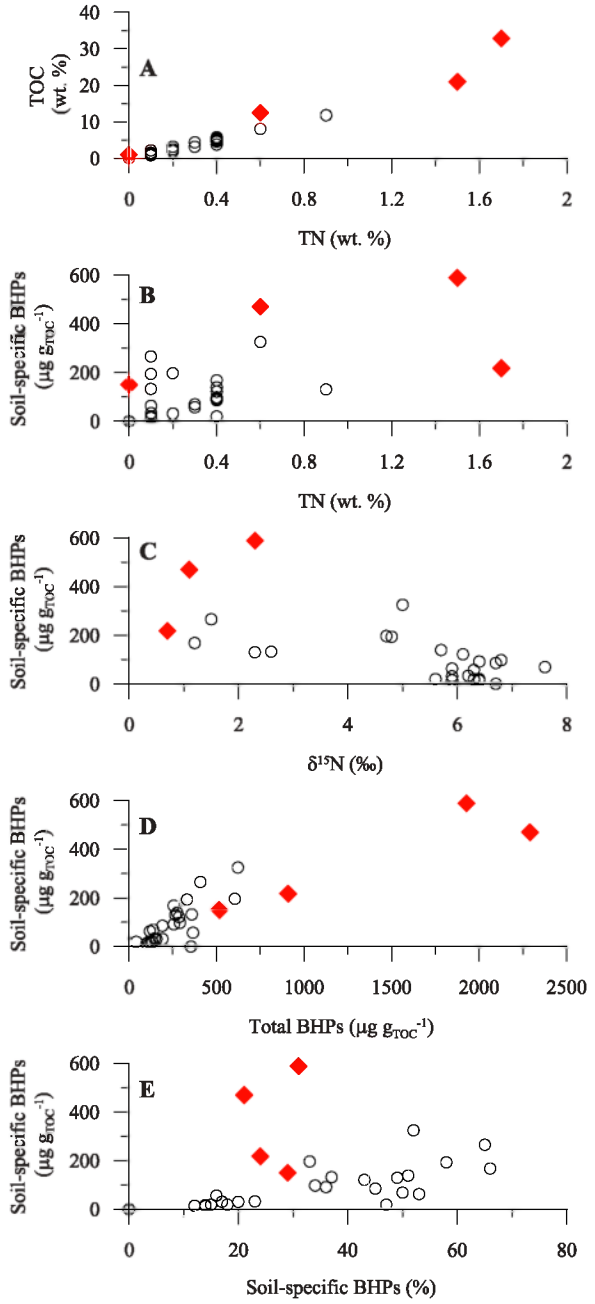

Figure 2. Comparison of the concentration of the soil-specific BHPs in μg g_{TOC}^{-1} with (a) TOC in wt. % [*Kim et al.*, 2010], (b) TN in wt. %, (c) δ^{15}N in ‰, (d) total BHPs in μg g_{TOC}^{-1}, and (e) soil-specific BHPs in %. Red diamonds and black circles indicate peat and soil samples, respectively.

response of the acetylated 5α-pregnane-3β,20β-diol internal standard added prior to derivatisation. Averaged relative response factors (from a suite of five acetylated BHP standards) were used to adjust the BHP peak areas relative to that of the internal standard where BHPs containing one or more N atoms give an averaged response approximately 12 times that of the standard and compounds with no N atoms give a response approximately 8 times that of the standard.

3.4. Statistical Analysis

[8] The relationships between environmental and geochemical parameters were assessed using redundancy analysis (RDA), using the Brodgar v.2.5.2 (www.brodgar.com) software package. Multiple colinearity between environmental variables was examined using variance inflation factors (VIFs). Large VIFs (>50) indicate that a variable is highly correlated with other variables, and thus contributes little information to the ordination. Preliminary ordinations revealed that altitude had a high VIF value. Therefore, the altitude was excluded in the final redundancy analysis. However, statistical analysis including altitude yielded similar results (data not shown).

4. Results

[9] The TOC and TN contents varied between 0.1 and 33 wt. % and between < 0.1 and 1.7 wt. %, respectively, with higher values found in peats (Figure 2a and Table 1). TN contents were only significant for soils near the source of the Têt River (Figure 3). The δ^{15}N values of the soils ranged from 0.7 to 7.6 ‰ with distinctively depleted δ^{15}N values in peats (Figure 2c).

[10] In total, 19 different BHP structures were identified in the investigated peat and soil samples (Table 2). The concentration of total BHPs ranged from 42 to 2289 μg g_{TOC}^{-1} (Figure 2d). Bacteriohopanetetrol (BHT; Ia) and aminotriol (Ib) were detected in all the samples (Table 3). BHT cyclitol ether (Ih) was also common but not detected in all the samples and was only observed in the surface sample at site TESO36. These source-unspecific BHPs (Table 2) contributed 26 to 92% of total BHPs (Figures 4 and 5).

[11] Usually, 1 to 4 of the "soil-marker" BHPs were present in all analyzed samples, except for the topsoil (TESO35) collected near to the river mouth. The concentration of soil-specific BHPs varied between 0 and 589 μg g_{TOC}^{-1} (Figure 4b). The proportion of soil-specific BHPs relative to total BHPs reached up to 66% (Figure 4e). In general, the concentrations of total and soil-specific BHPs were higher in the upper layers than in the deeper layers down the soil profiles (Figure 3); however,

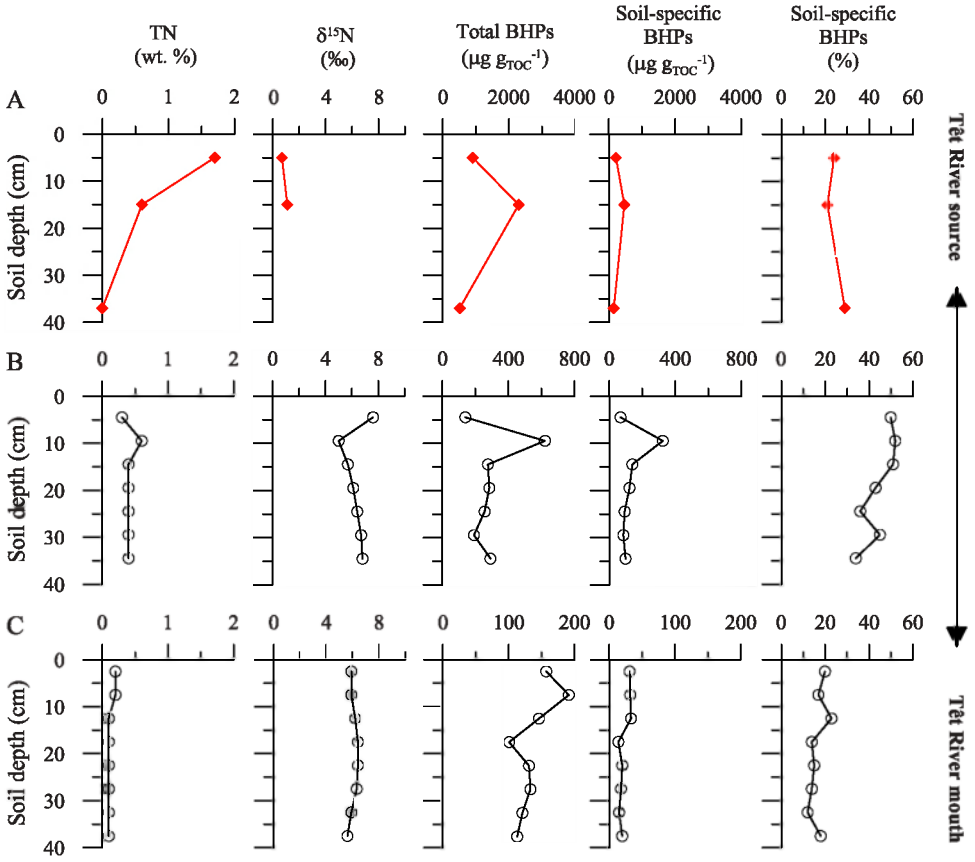

Figure 3. Bulk geochemical (TN in wt. % and δ^{15}N in ‰) and BHP parameters of soil depth profiles collected from the source area and the river mouth of the Tết River: (a) TESO2, (b) TESO5, and (c) TESO36. Red diamonds and black circles indicate peat and soil samples, respectively.

the proportion of soil-specific BHPs relative to the total BHPs did not clearly follow those of the concentrations of total and soil-specific BHPs.

5. Discussion

5.1. Occurrence and Abundance of Soil-Specific BHPs

[12] As observed in previous studies of soils [*Cooke et al.*, 2008a; *Xu et al.*, 2009; *Rethemeyer et al.*, 2010], BHPs in Tết soils are dominated by non-source-specific BHT, aminotriol, and BHT cyclitol ether (Figure 4). In contrast, contributions of BHPs from methanotrophic and likely phototrophic sources, including cyanobacteria and purple non-sulfur bacteria (Figure 4) are relatively minor. Despite their low abundances, cyanobacterial BHPs are commonly found in Tết peats and soils, but absent in deeper soil depths at the river mouth. This is in good agreement with recent studies, showing that the diversity of organisms capable of BHP biosynthesis is greater in terrestrial rather than marine influenced systems [e.g., *Pearson et al.*, 2009] and more specifically that BHP production in marine cyanobacteria seems to be uncommon [*Pearson et al.*, 2007; *Talbot et al.*, 2008].

[13] The methanotrophic bacteria markers aminotetrol (Ie) and aminopentol (If) were only observed at a small number of sites (Table 3) with the most diagnostic structure (aminopentol) [*Cvejic et al.*, 2000a] only present at 4 sites including the upper layers of the peat core (TESO2) and in topsoil TESO32. It was also observed in the deepest layer of site (TESO36) where it likely reflects preservation of a fossilized signature as it indicates an aerobic process which is unlikely to occur only in the deeper and anoxic part of the soil profiles.

[14] The soil-specific BHPs (adenosylhopane, adenosylhopane-type 1, 2-methyl adenosylhopane, and 2-methyl adenosylhopane-type 1) are common in all the samples but the topsoil (TESO35) collected near the river mouth. Strikingly, the con-

Table 2. BHPs Identified in the Investigated Têt Soil Samples[a]

Abbreviated Name	Structure[b]	Base Peak *m/z*	Known Source Organisms	References[c]
BHT	Ia	655	various	1–4, 6–8, 10, 11, 17, 19–21, 23–25, 27–29
2-methyl BHT	IIa	669	Cyanobacteria, *Rhodopseudomonas palustris*	2, 23, 29, 32
Unsaturated aminotriol	IIIb or IVb	712	*Rhodopseudomonas palustris*	27
Aminotriol	Ib	714	various	3–5, 7, 12, 13, 15, 16, 18, 20, 22, 25, 27, 28
2-methyl aminotriol	IIb	728	Cyanobacteria	31
Adenosylhopane	Ic	746	Purple nonsulfur bacteria, *Nitrosomonas europea, Bradyrhizobium japonicum*	4, 7, 14, 16, 27, 22
2-methyl adenosylhopane	IIc	760	*Bradyrhyzobium japonicum*	27
Adenosylhopane-type 1	Id	761	Purple nonsulfur bacteria	27
Aminotetrol	Ie	772	Methanotrophs, *Desulfovibrio sp.*	3, 12, 13, 25, 30
2-methyl adenosylhopane-type 1	IId	775	none	
Aminopentol	If	830	Type I methanotrophs	5, 13, 25
Unsaturated BHT pentose	IIIg or IVg	941	Cyanobacteria	31
BHT pentose	Ig	943	Cyanobacteria	31
2-methyl BHT pentose	IIg	957	Cyanobacteria	31
BHT cyclitol ether	Ih	1002 (C)	various	6–11, 16, 19, 26–28
BHT glucosamine	Ii	1002 (G)	various	8, 11, 19
BHpentol cyclitol ether	Ij	1060	various	10, 26–28
BHhexol cyclitol ether	Ik	1118	none	
2-methylBHhexol cyclitol ether	IIk	1132	none	

[a]BHT, bacteriohopanetetrol; BH, bacteriohopane.
[b]Structures in Appendix A.
[c]Related references: 1, *Berry et al.* [1991]; 2, *Bisseret et al.* [1985]; 3, *Blumenberg et al.* [2006]; 4, *Bravo et al.* [2001]; 5, *Cvejic et al.* [2000a]; 6, *Cvejic et al.* [2000b]; 7, *Flesch and Rohmer* [1988]; 8, *Flesch and Rohmer* [1989]; 9, *Herrmann et al.* [1996]; 10, *Joyeux et al.* [2004]; 11, *Knani et al.* [1994]; 12, *Neunlist and Rohmer* [1985a]; 13, *Neunlist and Rohmer* [1985b]; 14, *Neunlist and Rohmer* [1985c]; 15, *Neunlist et al.* [1985]; 16, *Neunlist et al.* [1988]; 17, *Peiseler and Rohmer* [1992]; 18, *Poralla et al.* [2000]; 19, *Renoux and Rohmer* [1985]; 20, *Rohmer* [1993]; 21, *Rosa-Putra et al.* [2001]; 22, *Seemann et al.* [1999]; 23, *Simonin et al.* [1996]; 24, *Sinninghe Damsté et al.* [2004]; 25, *Talbot et al.* [2001]; 26, *Talbot et al.* [2003c]; 27, *Talbot et al.* [2007a]; 28, *Vilcheze et al.* [1994]; 29, *Zhao et al.* [1996]; 30, *Zhou et al.* [1991]; 31, *Talbot et al.* [2008]; 32, *Rashby et al.* [2007].

centrations of soil-specific BHPs are highest in peats with depleted $\delta^{15}N$ values (Figure 2). The depleted $\delta^{15}N$ values in peats therefore suggest that soil-specific BHP production is favored by the growth of N_2-fixing bacteria. Adenosylhopane (Ic) and its methylated homologue (IIc) are, for instance, found in the nitrogen-fixing bacterium *Bradyrhizobium japonicum* [*Bravo et al.*, 2001; *Talbot et al.*, 2007a]. Therefore, the Têt data suggest that *B. japonicum* and/or yet other unknown N_2-fixing bacteria are one of the major sources of soil-marker BHPs in acidic environments.

5.2. Environmental Factors Controlling Soil-Specific BHP Productions

[15] In order to assess which environmental factors determine the concentrations of total and soil-specific BHPs normalized to TOC, the number of BHP compounds detected, and the percentage of the soil-specific BHPs relative to the total BHPs in the Têt watershed, we applied redundancy analysis on the data acquired. The variables pH, MAT, and precipitation (Table 1) explain 41% of the variation in the response (BHP parameters) variables (Table 4). Conditional effects (i.e., increase total sum of eigenvalues after including new variable) (Table 4) indicate that soil pH is the most important environmental factor, influencing the response variables and thus the production and occurrence of total and soil-specific BHPs. This response is in good agreement with recent culture studies showing that BHPs, 2-methyl compounds in particular, were produced in greater concentration by photosynthetic bacteria at lower pH [*Doughty et al.*, 2009; *Welander et al.*, 2009]. Therefore, our results support previous findings that BHPs are important in protecting cells from external stresses, such as pH. The pH and BHP abundance relationship is also similar to that of branched GDGT production [*Kim et al.*, 2010]. In general, the concentrations of total and soil-specific BHPs as well as the diversity of BHP producing organisms are higher in the Têt catchment when soil pH values are lower (Figures 6a–6c). However, there is no apparent relationship between the percentage of the soil-specific BHPs and soil pH values (Figure 6d).

Table 3. Individual BHP Data From the Têt Watershed in $\mu g\ g_{TOC}^{-1}$[a]

Sample Site	Material	Sampling Depth (cm)	Ia	IIa	IIIb or IVb	Ib	IIb	Ic	IIc	Id	IId	Ie	If	IIIg or IVg	Ig	IIg	Ih	Ii	Ij	Ik	IIk
TESO1	Soil	2–7	20	15	4	62	3	52	8	59	11	-	-	-	-	-	19	-	6	4	1
TESO2	Peat	0–10	190	60	-	170	5	110	3	90	15	10	13	-	27	-	190	7	9	8	2
TESO2	Peat	10–20	500	60	-	200	-	340	-	130	-	27	47	-	-	-	900	16	37	32	-
TESO2	Peat	37	160	17	-	69	-	150	-	-	-	-	-	-	-	-	120	-	-	-	-
TESO5	Soil	2–7	33	3	-	11	-	18	2	44	6	-	-	-	-	-	18	-	2	1	-
TESO5	Soil	7–12	62	50	-	57	3	100	8	180	37	2	-	9	6	-	94	-	4	8	1
TESO5	Soil	12–17	38	16	-	39	2	28	-	91	20	-	-	-	-	-	37	-	3	1	-
TESO5	Soil	17–22	38	10	2	38	2	36	2	69	15	-	-	-	-	-	66	-	3	3	-
TESO5	Soil	22–27	21	7	-	43	-	21	-	58	13	-	-	-	-	-	88	-	3	2	-
TESO5	Soil	27–32	22	5	-	28	1	23	-	54	9	-	-	-	-	-	44	-	3	3	-
TESO5	Soil	32–37	39	6	-	37	-	35	-	52	11	-	-	-	-	-	100	-	6	4	-
TESO15	Soil	1–5	190	13	-	17	-	90	4	32	6	-	-	-	-	-	6	-	-	-	-
TESO17	Soil	upper 10	19		-	3	-	16	-	4	-	-	-	-	-	-	-	-	-	-	-
TESO19	Soil	upper 10	110	12	-	16	-	130	14	80	42	4	-	-	-	-	-	-	-	-	-
TESO32	Soil	upper 10	160	19	-	25	-	57	-	-	-	8	13	-	-	-	83	-	-	-	-
TESO35	Soil	upper 10	280	30	-	43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TESO36	Soil	0–5	88	15	-	12	-	31	-	-	-	-	-	-	-	-	9	-	2	-	-
TESO36	Soil	5–10	130	14	-	15	-	32	-	-	-	-	-	-	-	-	-	-	-	-	-
TESO36	Soil	10–15	100	-	-	13	-	33	-	-	-	-	-	-	-	-	-	-	-	-	-
TESO36	Soil	15–20	75	-	-	12	-	14	-	-	-	-	-	-	-	-	-	-	-	-	-
TESO36	Soil	20–25	97	-	-	14	-	20	-	-	-	-	-	-	-	-	-	-	-	-	-
TESO36	Soil	25–30	110	-	-	5	-	18	-	-	-	-	-	-	-	-	-	-	-	-	-
TESO36	Soil	30–35	83	-	-	12	-	15	-	-	-	2	5	-	-	-	3	2	-	-	-
TESO36	Soil	35–40	85	-	-	4	-	20	-	-	-	-	3	-	-	-	-	-	-	-	-
TESO39	Soil	upper 10	38	-	-	12	-	46	-	11	6	3	-	-	-	-	2	-	1	1	-
TESO41	Soil	upper 10	84	14	-	30	-	110	9	60	15	10	-	-	-	-	-	-	-	-	-
TESO47	Soil	upper 10	50	18	-	14	-	80	8	70	10	-	-	-	-	-	3	3	-	-	-
TESO48	Soil	0–20	77	42	3	94	3	110	8	76	3	-	-	66	32	5	57	-	10	13	7
TESO49	Peat	0–10	240	78	37	290	-	370	34	151	34	-	-	-	-	-	640	-	27	26	-

[a]MAT indicates mean annual air temperature. Roman numerals refer to BHP structures shown in Appendix A and "-" means not detected.

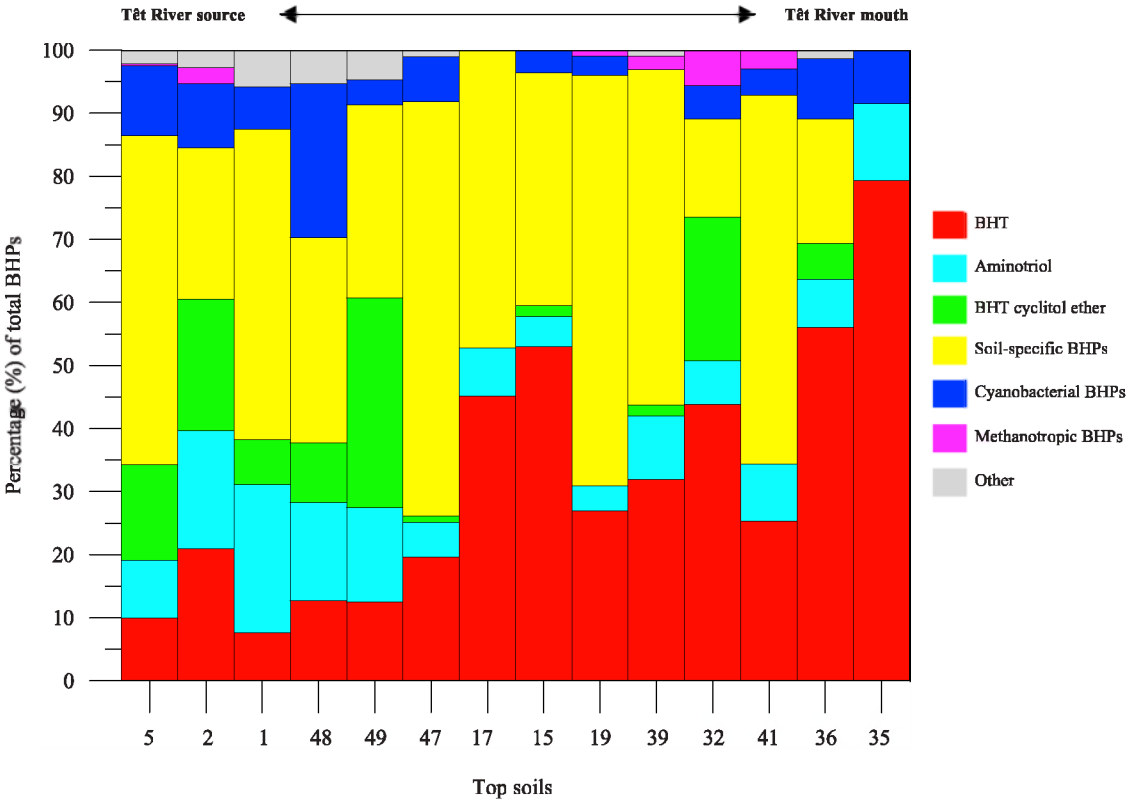

Figure 4. Distributions of individual or grouped BHPs relative to the total BHPs in % for topsoils. Cyanobacterial BHPs include 2-methyl BHT (IIa), 2-methyl aminotriol (IIb), unsaturated BHT pentose (IIIg or IVg), BHT pentose (Ig), and 2-methyl BHT pentose (IIg). Soil-specific BHPs include adenosylhopane (Ic), 2-methyl adenosylhopane (IIc), adenosylhopane-type (Id), and 2-methyl adenosylhopane-type (IId). Methanotrophic BHPs are the group of aminotetrol (Ie) and aminopentol (If). Numbers on the *x* axis correspond to TESO numbers in Table 1.

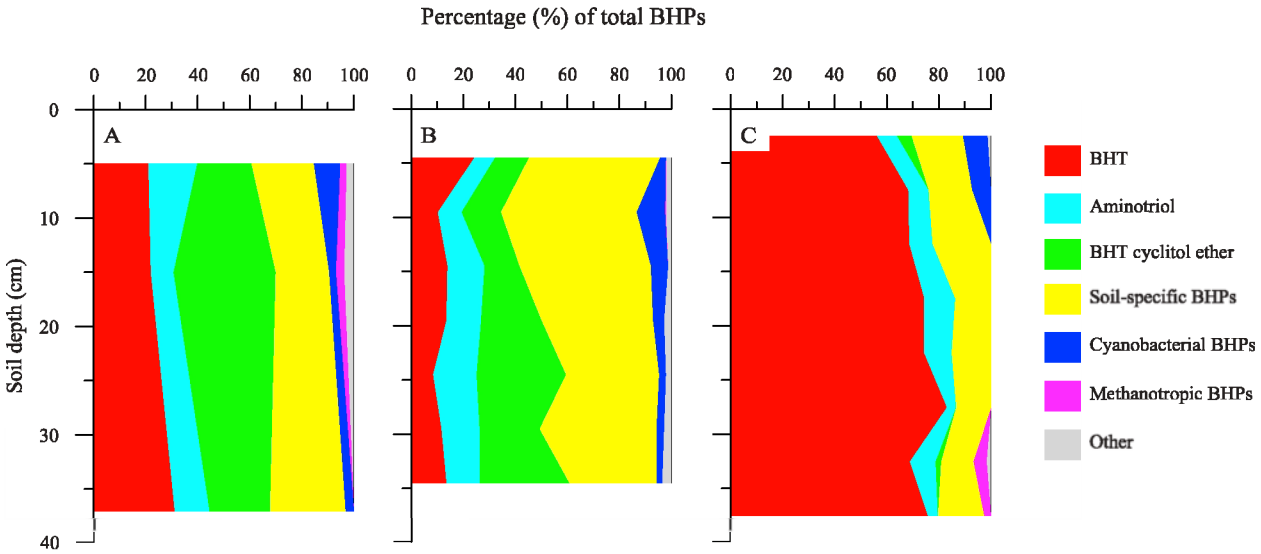

Figure 5. Distributions of individual or grouped BHPs relative to the total BHPs in % for soil depth profiles: (a) TESO2, (b) TESO5, and (c) TESO36.

Table 4. Numerical Output of a Redundancy Analysis Applied to the BHP Data[a] and Conditional Effects[b]

Axis	λ	λ as Cumulative %	λ as Cumulative % of Sum of All Canonical Eigenvalues
1	0.40	40	94
2	0.02	42	99
Order	**Explanatory Variable**	**Conditional Effects**	***p* Value**
1	**pH**	**0.35**	**0.005**
2	precipitation	0.04	0.200
3	MAT	0.03	0.385

[a]The sum of all canonical eigenvalues is 0.42 and the total variance is 1 (the variation explained by the first two axes). Eigenvalue (λ) is the standard deviation of the scores.

[b]Eigenvalue indicates the increase in explained variation due to adding an extra explanatory variable. Response variables used are the concentration of total BHPs, the concentration of soil-specific BHPs, the percentage of the soil-specific BHPs relative to the total BHPs, and the numbers of BHP compounds detected. Explanatory variables used are soil pH, mean annual air temperature (MAT), and precipitation. Significance level, $p < 0.05$. Conditional effects are total sum of eigenvalues after including new explanatory variable.

5.3. Comparison of Soil-Specific BHPs With Branched GDGTs

[16] The topsoil of TESO35 influenced by seawater contains virtually no soil-specific BHPs. Furthermore, the concentration of soil-specific BHPs from the TESO36 soil profile near the Têt River mouth are significantly lower than those from the TESO2 and TESO5 soil profiles upstream of the Têt River. Accordingly, our data generally support the initial hypothesis that soil-specific BHPs can be a useful tool to identify the transport of soil OM to marine sediments [*Talbot and Farrimond*, 2007; *Cooke et al.*, 2008b; *Rethemeyer et al.*, 2010].

[17] In order to further explore the potential of soil-specific BHPs as a soil OM tracer, we compared soil-specific BHPs with branched GDGT parameters, which are more established indicators of soil OM input from land to the ocean. The comparison of the soil-specific BHP concentrations with the branched GDGT concentrations (Figure 7a) does not show significant relationships for both peats ($r^2 = 0.5$, $p = 0.3$) and soils ($r^2 = 0.06$, $p = 0.24$). The comparison of BIT values with the soil-

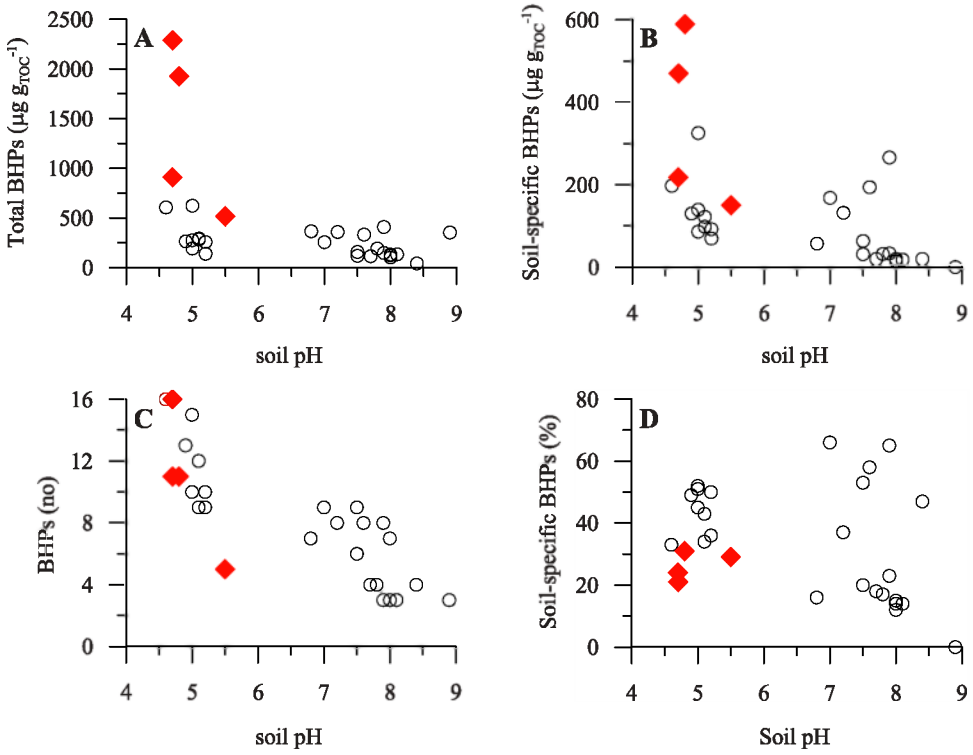

Figure 6. Scatterplots of soil pH values with (a) the concentration of total BHPs in μg g_{TOC}^{-1}, (b) the concentration of soil-specific BHPs in μg g_{TOC}^{-1}, (c) the numbers of BHP compounds detected, and (d) the percentage of the soil-specific BHPs relative to the total BHPs in % (see also Table 3). Red diamonds and black circles indicate peat and soil samples, respectively.

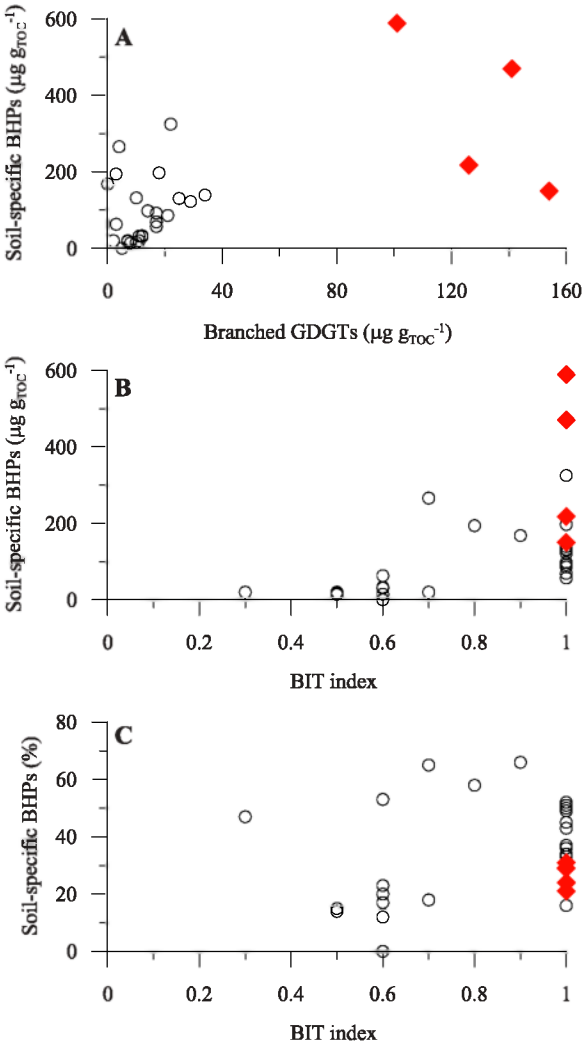

Figure 7. Comparisons of soil-specific BHPs and branched GDGTs: (a) the concentration of soil-specific BHPs in μg g_{TOC}^{-1} versus the summed concentration of branched GDGTs in μg g_{TOC}^{-1}, (b) the concentration of soil-specific BHPs in μg g_{TOC}^{-1} versus BIT values, and (c) the percentage of the soil-specific BHPs relative to the total BHPs in % versus BIT values. Red diamonds and black circles indicate peat and soil samples, respectively. The branched GDGT and BIT data were previously reported by *Kim et al.* [2010].

specific BHP concentrations (r^2 = 0.3, p = 0.003, Figure 7b) and the percentage of soil-specific BHPs relative to total BHPs (r^2 = 0.2, p = 0.03, Figure 7c) also show no significant correlations. Although more data from different environmental settings and statistical evaluations are clearly needed to validate the absence of relationship observed in the Têt River, our results imply that both biomarkers are produced by different organisms.

[18] Up to now, only planctomycetes, performing the anaerobic oxidation of ammonium [*Sinninghe Damsté et al.*, 2004], *Geobacter* species [*Fischer et al.*, 2005], and sulfate-reducing bacteria of the genus *Desulfovibrio* [*Blumenberg et al.*, 2006] have been shown to produce BHPs under strictly anoxic conditions. Therefore, it is consistent that both total and soil-specific BHPs are more abundant in the aerated zones than in the deeper anaerobic parts of soil profiles (Figure 3). This vertical distribution pattern is, to some degree, inverse to that of the branched GDGTs, which show higher abundances in deeper parts of the soil profiles [*Kim et al.*, 2010] and peats [*Weijers et al.*, 2007]. This is also in agreement with previous studies, suggesting that branched GDGTs are produced by anaerobic bacteria [*Weijers et al.*, 2006a]. Thus, soil-specific BHPs and branched GDGTs are likely produced by bacteria living in different ecological niches of the soil profile.

6. Conclusions

[19] BHP distributions have been analyzed from a range of soils and peats from 14 sites in the catchment of the River Têt. In all samples but one (TESO35) collected near the Têt River mouth, the previously proposed soil-marker BHPs (adenosylhopane and up to three structurally related compounds) were detected in proportions up to 60% relative to total BHPs, significantly higher than the previously reported global mean for soils of 28% [*Cooke et al.*, 2008b] although higher values have been reported in both Canadian soils [*Xu et al.*, 2009] and soils from Svalbard [*Rethemeyer et al.*, 2010]. Concentrations of soil-specific BHPs in peat environments have been shown to increase with lower $\delta^{15}N$ values, suggesting that N_2-fixing bacteria are one of the major sources of BHPs including soil-marker BHPs in peats. Soil pH is also a major factor controlling the diversity and concentration of total and soil-marker BHPs similar to previous observations for branched GDGTs and in agreement with laboratory culture studies looking at BHP biosynthesis under changing pH regimes. Further studies are clearly required to determine what, if any other environmental factors, determine BHP diversity, concentration and preservation including e.g., temperature, a wider range of pH values, salinity, and pressure such as pCO_2.

[20] The concentrations of soil-specific BHPs from the soil profile near the Têt River mouth are signif-

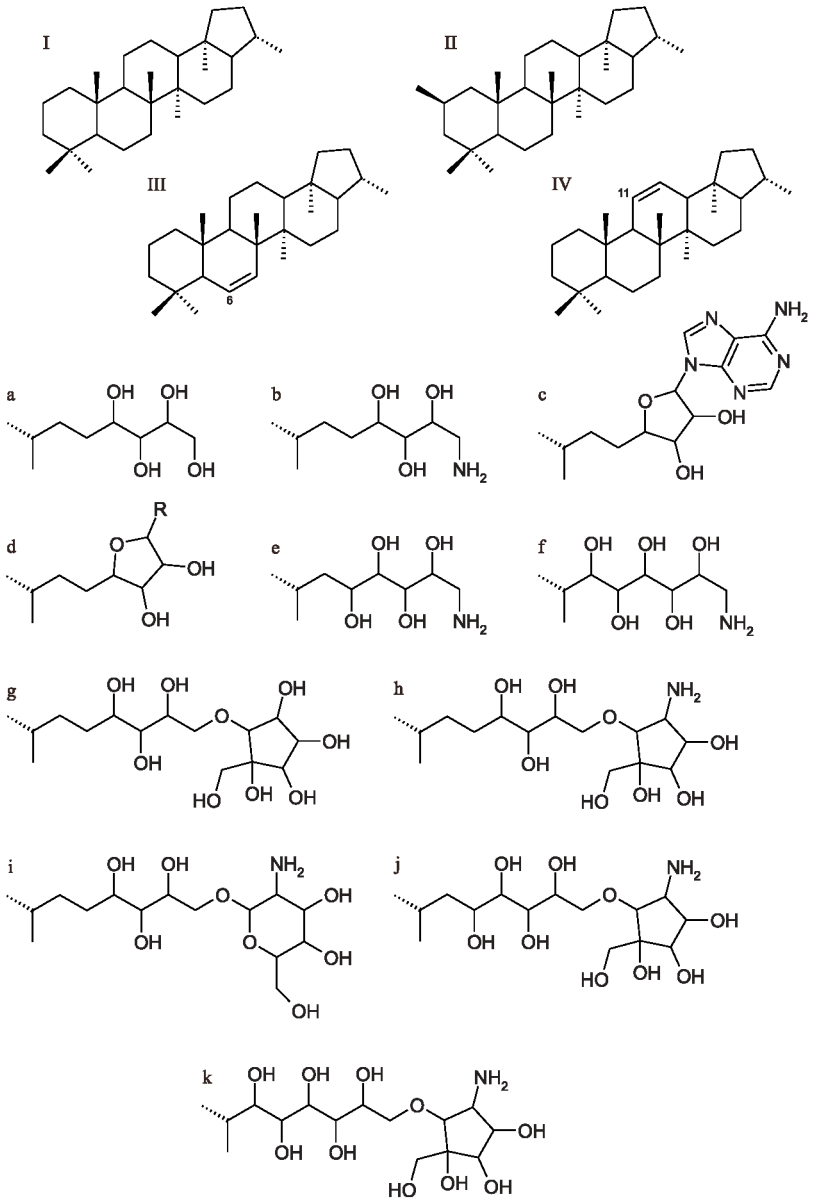

Figure A1. Structures of BHPs found in soil samples from the Têt watershed.

icantly lower than those of the soil profiles upstream of the Têt River. This supports the initial hypothesis that soil-specific BHPs can be useful biomarkers for tracking the input of soil OM from land to aquatic environments [*Talbot and Farrimond*, 2007]. However, there is no clear correlation between the percentage of soil-specific BHPs relative to total BHPs and the BIT index likely due to the different ecological niches occupied by the major sources organisms (aerobic topsoil versus deeper anaerobic horizons). More work is needed to extend our limited knowledge on production and occurrence of both soil-specific biomarkers in various environmental settings.

Appendix A

[21] BHP structures in the soil samples are investigated in this study (Figure A1). The stereochemistry indicated was previously determined from NMR studies, but other configurations may be possible.

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