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Occurrence and distribution of tetraether membrane lipids in soils: Implications for the use of the TEX_{86} proxy and the BIT index

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Abstract

A diverse collection of globally distributed soil samples was analyzed for its glycerol dialkyl glycerol tetraether (GDGT) membrane lipid content. Branched GDGTs, derived from anaerobic soil bacteria, were the most dominant and were found in all soils. Isoprenoid GDGTs, membrane lipids of Archaea, were also present, although in considerably lower concentration. Crenarchaeol, a specific isoprenoid membrane lipid of the non-thermophilic Crenarchaeota, was also regularly detected and its abundance might be related to soil pH. The detection of crenarchaeol in nearly all of the samples is the first report of this type of GDGT membrane lipid in soils and is in agreement with molecular ecological studies, confirming the widespread occurrence of non-thermophilic Crenarchaeota in the terrestrial realm. The fluvial transport of crenarchaeol and other isoprenoid GDGTs to marine and lacustrine environments could possibly bias the BIT index, a ratio between branched GDGTs and crenarchaeol used to determine relative terrestrial organic matter (TOM) input. However, as crenarchaeol in soils is only present in low concentration compared to branched GDGTs, no large effect is expected for the BIT index. The fluvial input of terrestrially derived isoprenoid GDGTs could also bias the TEX_{86}, a proxy used to determine palaeo surface temperatures in marine and lacustrine settings and based on the ratio of cyclopentane-containing isoprenoid GDGTs in marine and lacustrine Crenarchaeota. Indeed, it is shown that a substantial bias in TEX_{86}-reconstructed sea and lake surface temperatures can occur if TOM input is high, e.g. near large river outflows.
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1. Introduction

Culture-independent molecular ecological techniques have shown that the Archaea, comprising the Kingdoms Crenarchaeota, Euryarchaeota and Korarchaeota, inhabit a widespread diversity of environments from extremophilic to mesophilic settings. In mesophilic environments, Archaea occupy

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diverse settings such as marine and lacustrine water columns and sediments (e.g. DeLong et al., 1994; MacGregor et al., 1997; Schleper et al., 1997; Vetriani et al., 1999; Jurgens et al., 2000; Karner et al., 2001; Keough et al., 2003), peat bogs, wetlands and soils (e.g. Buckley et al., 1998; Ochsenreiter et al., 2003; Sizova et al., 2003; Kotsyurbenko et al., 2004) and the deep subsurface (Takai et al., 2001). Despite being ubiquitous, only a limited number of archaeal cultures is available, mostly thermophiles and methanogens, one 'symbiont culture' of a mesophilic Crenarchaeota: *Cenarchaeum symbiosum*, an archaeon living in symbiosis with the marine sponge *Axinella mexicana* (Preston et al., 1996) and a nitrifying crenarchaeote isolated from a sea aquarium (Könneke et al., 2005).

From most of these cultures it is known that Archaea synthesize characteristic isoprenoid glycerol dialkyl glycerol tetraether (GDGT) membrane lipids. Archaeal GDGT membrane lipids can, therefore, provide additional information on the presence and diversity of the archaeal community. Archaeal GDGT membrane lipids have been found predominantly in wetland environments, like peat bogs (Pancost et al., 2000; Schouten et al., 2000; Pancost and Sinninghe Damsté, 2003; Weijers et al., 2004), where they are most likely derive from methanogenic Euryarchaeota (Pancost et al., 2000), and in marine environments, where they occur ubiquitously (Schouten et al., 2000). More recently, archaeal GDGT lipids have also been reported in sediments of some large lakes (Powers et al., 2004). In marine and lacustrine environments, the GDGT lipids are most likely derived from non-thermophilic pelagic Crenarchaeota (i.e. group 1.1 Crenarchaeota; DeLong, 1998), since they always contain a unique GDGT lipid, crenarchaeol (**VI**; see Appendix for structures), containing an additional cyclohexyl moiety. This compound is considered a biomarker for the group 1.1 Crenarchaeota as it has only been found in *C. symbiosum* (Sinninghe Damsté et al., 2002c) and not in thermophilic or methanogenic Archaea. Another recently discovered group of GDGT membrane lipids, containing branched instead of isoprenoid alkyl chains, is found mainly in peat bogs (Schouten et al., 2000; Sinninghe Damsté et al., 2000a; Pancost and Sinninghe Damsté, 2003; Weijers et al., 2006), but also in coastal marine sediments (Hopmans et al., 2004) and lake sediments (Powers et al., 2004). Because of the branched alkyl chains and the bacterial 1,2-di-*O*-alkyl-*sn*-glycerol stereochemical configuration at C-2 in the glycerol backbone, those tetraethers are most likely of bacterial rather than archaeal origin (Weijers et al., 2006).

Based on these GDGT membrane lipids, two proxies have recently been developed: the TEX_{86} sea surface temperature (SST) proxy (Schouten et al., 2002), recently also adapted for application to lakes (Powers et al., 2004), and the branched vs. isoprenoid tetraether (BIT) index, a proxy for the relative fluvial input of terrestrial organic matter (TOM) in the marine environment (Hopmans et al., 2004). The TEX_{86} SST proxy is based on the relative distribution of cyclopentane-containing isoprenoid GDGT lipids (**II**–**IV**, **VI**′) in the membranes of non-thermophilic pelagic Crenarchaeota. This distribution pattern has been shown to be primarily dependent on growth temperature (Wuchter et al., 2004). The BIT index is the ratio between crenarchaeol (**VI**) and three branched GDGT lipids (**VII**–**IX**) in marine and lacustrine sediments. Based on a rapid decrease in the concentration of branched GDGTs with increasing distance from the Congo River outflow (eastern tropical Atlantic; Hopmans et al., 2004) and the detection of branched GDGTs in river water samples from the river Rhine, The Netherlands (Herfort et al., 2006), it is assumed that branched GDGTs are only terrestrially produced and are fluvially transported to lakes and oceans. The fact that branched GDGTs are detected in many different coastal areas suggests that these compounds are ubiquitous in the terrestrial realm. Branched GDGTs on land have, however, only been reported in a few west European peat bogs (Schouten et al., 2000; Sinninghe Damsté et al., 2000a; Pancost and Sinninghe Damsté, 2003; Weijers et al., 2006) and one Dutch soil (Hopmans et al., 2004). In fact, the occurrence of both branched and isoprenoid GDGT membrane lipids in soils has not been investigated in any detail. So far, Gattinger et al. (2003) have reported the presence of GDGTs **I**–**V** in one bulk soil sample composed of different soil types and Hopmans et al. (2004) have detected branched GDGTs **VIII** and **IX** in a deciduous forest soil from The Netherlands. The aim of the current study was, therefore, to investigate the distribution of GDGT membrane lipids in soils in more detail and to discuss their possible implications for the use of the BIT index and TEX_{86} proxy. To achieve this, a wide variety of globally distributed soils was investigated for GDGT membrane lipid content.

2. Material and methods

2.1. Sample collection

To cover a wide diversity of soils, 58 samples from 26 globally distributed locations with different land use and vegetation patterns, and different organic contents (C_{org}) and pH values, were obtained (Table 1). Most were collected from the World Soil Database collection of the International Soil Research and Information Centre (ISRIC) in Wageningen, The Netherlands. They were obtained with hand auguring equipment during different field trips over the last few decades. Although not sterilized, they had been stored dry and in the dark at room temperature and any biological activity since is therefore assumed to be negligible. Samples of two soils from the island of Texel (The Netherlands) and one from Scotland were taken in summer 2000 and summer 2004, respectively, and freeze dried prior to further preparation.

2.2. Sample preparation

The samples were extracted with a mixture of dichloromethane (DCM):methanol (9:1, v/v, ×3, 5 min each) using an accelerated solvent extractor (ASE 200, Dionex) at 100 °C and ca. 7.6×10^6 Pa. Each total extract was evaporated to near dryness using a rotary evaporator under near vacuum and separated over an activated Al_2O_3 column using hexane:DCM (1:1, v/v) and DCM:methanol (1:1, v/v). The latter fraction, containing the GDGTs, was dried under a continuous N_2 flow, ultrasonically dissolved in a hexane:propanol (99:1, v/v) solution at a concentration of ca. 2 mg ml^{-1} and filtered over a 0.45 μm PTFE filter (Alltech) prior to analysis.

Table 1
Land use, location, TOC and pH values of soils

Sample	Landuse/vegetation	Latitude	Longitude	Altitude (m)	Depth (cm)[a]	C org[b]	pH[b]
Alaska-17	Alluvial terrace	64:52 N	147:50 W	ca. 200	0–28 (2)	4.0–3.1	7.1–7.3
Cameroon-1	Coffee farm	4:14 N	9:20 E	1200	0–55 (2)	3.0–0.5	6.4–6.1
Canada-17	Cropland (cleared forest)	49:57 N	98:11 W	256	0–70 (4)	n.d.[c]	n.d.
France-15	Semi-natural grassland, grazed	45:03 N	2:33 E	1080	0–90 (4)	6.0[d]	4.7–5.1
Gabon-1	Tropical evergreen forest	0:31 N	12:48 E	530	0–65 (3)	3.3–0.6	3.3–4.2
Gabon-2	Savannah with parts of forest	1:31 S	14:07 E	640	6–15 (1)	0.2	5.3
Gabon-3	Herbs and grasses after burning	1:41 S	13:35 E	350	0–25 (2)	5.3–3.5	4.7–4.8
Gabon-4	Grassland	2:13 S	11:32 E	215	0–19 (2)	2.7–1.0	5.3–5.2
Gabon-5	Semi-deciduous shrub	2:21 S	11:23 E	150	0–20 (1)	2.2	5.1
Gabon-6	Grassland	0:31 S	10:17 E	150	0–25 (2)	2.5–0.6	5.9–5.6
Ghana-2	Coastal savannah grassland	5–7 N	ca. 0	27	0–24 (2)	0.3–0.2	6.0–5.7
Greece-13	Deciduous forest	40:30 N	23:33 E	600	0–3 (1)	n.d.	5.4
Greenland-05	Tundra	65:37 N	37:40 W	ca. 500	0–35 (2)	n.d.	n.d.
Hawaii-10	Semi-natural grassland, grazed	22:4 N	159:24 W	180	0–64 (2)	4.0–2.2	5.1–5.4
Iceland-6	Heath and mosses, grazed	65:21 N	20:53 W	ca. 100	5–30 (2)	6.6–5.7	6.1–6.5
Ireland-9	Grazed shrubland	53:54 N	7:48 W	50	0–40 (2)	24.0–0.6	3.8–4.6
Italy-1	Grazed woodland	39:40 N	16:09 E	500	0–10 (1)	2.9	6.2
Nigeria-15	Grazed grassland in delta	5:18 N	6:38 E	2	0–55 (4)	3.1–0.1	4.4–5.0
Nigeria-19	Semi-deciduous forest	6:37 N	3:30 E	35	0–60 (2)	2.2–0.6	7.3–7.4
Scotland	Grazed pasture grassland	55:0 N	3:6 W	0	0–10 (1)	n.d.	n.d.
South-Africa-7	Grassland with acia trees	29:47 S	30:41 E	765	0–60 (3)	1.8–0.7	5.5
Spain-7	Semi-natural grassland, grazed	38:59 N	6:20 W	260	0–15 (2)	3.9–0.7	5.5–5.1
Sweden-4	Grassland, cultivated pasture	55:49 N	14:04 E	80	0–35 (2)	0.92	7.7
Texel-1	Coniferous forest	53:04 N	4:44 E	5	0–10 (1)	n.d.	n.d.
Texel-2	Deciduous forest	53:04 N	4:44 E	5	0–10 (1)	n.d.	n.d.
Zaire-1	Evergreen forest	0:52 N	24:28 E	440	0–150 (5)	n.d.	3.9–4.3
Zaire-2	Rubber plantation	0:46 N	24:26 E	460	0–30 (2)	1.26	4.1–4.4

[a] In brackets number of depth intervals for which a sample is analyzed.
[b] Range from uppermost to lowermost depth interval.
[c] n.d., not determined.
[d] Value for upper layer.

2.3. Analysis

Samples were analyzed using high performance liquid chromatography/atmospheric pressure chemical ionization-mass spectrometry (HPLC/APCI-MS), according to Hopmans et al. (2000) with minor modifications. Analyses were performed with an Agilent 1100 series/1100 MSD series instrument, equipped with an auto-injection system and HP-Chemstation software. Separation was achieved on a Prevail Cyano column (150 mm × 2.1 mm, 3 μm; Alltech). The flow rate of the hexane:propanol (99:1, v/v) eluent was 0.2 ml min^{-1}, isocratic for the first 5 min, and thereafter with a linear gradient to 1.8% propanol in 45 min. Injection volume was 10 μl. In order to enable detection of low concentrations of GDGTs, MS analysis was performed in the single ion monitoring (SIM) mode. Quantification was achieved by integrating the peak areas in the $[M+H]^+$ ion trace (i.e. protonated molecule) and comparing these to an external calibration curve prepared with known amounts of pure crenarchaeol (**VI**). A correction was made for the branched GDGTs (**VII**–**IX**), as their molecular mass is lower than that of crenarchaeol (**VI**).

BIT indices were calculated following the equation of Hopmans et al. (2004):

$$\text{BIT index} = \frac{[\mathbf{VII} + \mathbf{VIII} + \mathbf{IX}]}{[\mathbf{VII} + \mathbf{VIII} + \mathbf{IX}] + [\mathbf{VI}]} \quad (1)$$

The TEX_{86} was calculated as follows (Schouten et al., 2002):

$$TEX_{86} = \frac{[\mathbf{III} + \mathbf{IV} + \mathbf{VI'}]}{[\mathbf{II} + \mathbf{III} + \mathbf{IV} + \mathbf{VI'}]} \quad (2)$$

The TEX_{86} was converted to temperature according to the empirically derived formula given by Schouten et al. (2002):

$$T\ (^{\circ}\mathrm{C}) = \frac{TEX_{86} - 0.28}{0.015} \quad (3)$$

An alternative TEX_{86} proxy, the TEX'_{86}, applied by Sluijs et al. (2006) to reduce the influence of terrestrially derived isoprenoid GDGTs, is defined as:

$$TEX'_{86} = \frac{[\mathbf{III} + \mathbf{VI'}]}{[\mathbf{II} + \mathbf{III} + \mathbf{VI'}]} \quad (4)$$

The TEX'_{86} was converted to temperature with the formula given by Sluijs et al. (2006):

$$T'\ (^{\circ}C) = \frac{TEX'_{86} - 0.20}{0.016} \quad (5)$$

3. Results

The isoprenoid and branched GDGT membrane lipid content of the soils was determined by HPLC/APCI-MS analysis (Table 2). Branched GDGTs dominate, especially GDGT **IX** and to a lesser extent **VIII** (e.g. Fig. 1), which range in concentration from 10 to 1000 ng g^{-1} dry weight soil. Branched GDGT **VII** is, however, only present in considerably lower concentration (0–100 ng g^{-1}). The isoprenoid GDGTs **I** and **VI** (crenarchaeol) are virtually always present and concentrations vary roughly from 1 to 100 ng g^{-1}. The cyclopentane-containing isoprenoid GDGTs **II**–**IV** are usually present in low amounts and sometimes below detection limit, with concentrations between 0 and 10 ng g^{-1}. GDGT **VI′**, a regio-isomer of **VI** (crenarchaeol), was detected in about half of the soils at a concentration generally <2 ng g^{-1}. Only traces of GDGT **V** seem to be present and it could not be quantified as GDGT **VI** (crenarchaeol) coelutes and the isotope peaks of crenarchaeol interfere in the quantification.

At first sight, concentrations of GDGTs seem to be lower in soils from tropical areas (Table 2). It should be noticed, however, that the concentrations are reported in ng g^{-1} dry weight soil. If they are calculated as μg g^{-1} TOC, the difference is less apparent, as TOC contents of some of the non-tropical soils can be as high as 24% (e.g. Ireland), whereas that of tropical soils is lower (Table 1).

For three locations (Zaire-1, France-15 and Nigeria-15) a GDGT concentration profile with depth, down to 150, 90 and 55 cm, respectively, was obtained (Fig. 2a–c). Although there are differences in the absolute amounts of the different branched GDGTs (**VII**–**IX**), their distribution pattern is the same in each depth interval and so their concentrations are summed and plotted in one graph. Concentrations of the branched GDGTs (**VII**–**IX**) are clearly highest in the uppermost horizon and decrease rapidly with depth at all three locations. Also the profiles of GDGT **VI** (crenarchaeol) show a decrease from the top horizon downward, although in the Zairian profile (Fig. 2a) concentrations stabilize or even increase slightly again in the deeper intervals. Concentrations of GDGT **I** generally decrease with depth, although those in the Zairian profile seem to increase slightly again in the deeper part (Fig. 2a) and in the Nigerian profile a clear peak in the second depth interval is visible (Fig. 2c). Similar to the branched GDGTs,

Table 2
Concentration (ng g^{-1} dry weight soil) of GDGT lipids with BIT index

Sample	Depth (cm)	I	II	III	IV	VI	VI′	VII	VIII	IX	BIT index
Alaska-17	0–20	22	9.1	7.0	2.7	34	1.1	17	33	15	0.66
	20–28	2.8	3.1	2.1	0.9	9.7	0.2	25	43	15	0.90
Cameroon-1	0–15	1.0	0.3	0.6	0.0	8.6	1.7	0.6	5.6	26	0.82
	38–55	1.8	1.8	3.5	2.9	0.2	0.0	0.0	0.6	6.1	0.98
Canada-17	0–15	10	3.1	3.9	2.7	25	2.9	19	35	13	0.73
	15–22	6.7	2.5	3.7	2.3	20	2.6	18	31	11	0.74
	44–64	2.3	1.3	2.0	1.5	10	1.2	26	44	17	0.89
	64–70	3.4	2.5	4.4	2.8	22	1.7	55	80	32	0.88
France-15	0–8	244	38	22	5.6	106	5.3	165	724	722	0.94
	8–32	57	24	29	7.6	35	1.9	29	169	188	0.92
	32–50	46	25	25	4.4	12	1.3	25	128	139	0.96
	50–90	30	18	23	4.5	3.8	0.6	4.7	28	46	0.95
Gabon-1	0–7	10	3.9	5.7	4.3	16	6.1	1.8	18	162	0.94
	7–20	3.4	1.2	1.5	1.4	3.6	1.4	0.3	4.3	53	0.95
	50–65	1.4	1.0	1.4	2.1	0.5	0.1	0.0	0.6	6.6	0.95
Gabon-2	6–15	0.4	0.1	0.1	0.1	0.3	0.0	0.0	0.1	6.1	0.96
Gabon-3	0–10	1.4	0.6	0.8	0.4	1.9	0.1	0.0	2.9	155	0.99
	10–25	1.3	0.7	0.9	0.6	0.9	0.1	0.0	0.5	32	0.98
Gabon-4	0–10	1.8	0.4	0.3	0.2	2.2	0.1	0.0	2.3	62	0.97
	10–19	0.7	0.3	0.2	0.2	0.1	0.0	0.0	0.5	19	1.00
Gabon-5	0–20	2.5	0.4	0.3	0.2	0.1	0.0	0.0	0.7	25	1.00
Gabon-6	0–5	2.2	1.7	2.3	2.0	26	1.5	0.5	6.4	51	0.74
	10–25	1.1	0.9	1.0	0.6	2.5	0.4	0.0	0.7	22	0.92
Ghana-2	0–10	0.6	0.2	0.7	0.3	5.4	0.3	0.0	0.2	17	0.80
	10–24	0.0	0.1	0.2	0.1	2.1	0.1	0.0	0.0	8.5	0.84
Greece-13	0–3	97	0.0	0.0	0.0	4.5	0.0	21	98	123	0.98
Greenland-05	0–5	0.0	0.0	0.0	0.0	0.9	0.0	16	45	35	0.99
	5–35	1.1	0.0	0.0	0.0	0.8	0.0	19	89	99	1.00
Hawaii-10	0–35	2.9	1.5	1.7	1.0	6.8	0.5	0.5	10	144	0.96
	35–64	5.4	4.6	7.4	5.3	1.2	0.1	0.4	6.9	60	0.98
Iceland-6	5–15	17	6.0	4.8	3.2	21	0.0	217	728	459	0.99
	17–30	79	8.8	13	2.7	51	1.2	471	1261	732	0.98
Ireland-9	0–7	99	10	15	13	7.0	0.0	102	971	1389	1.00
	7–40	16	1.4	1.4	0.9	0.9	0.0	3.4	42	79	0.99
Italy-1	0–10	5.1	1.2	1.0	0.2	0.9	0.1	5.0	23	24	0.98
Nigeria-15	0–12	4.1	1.9	2.2	2.4	0.4	0.0	0.0	1.9	92	1.00
	12–25	11	5.1	7.0	11	0.3	0.1	0.0	1.1	72	1.00
	25–37	2.1	1.5	2.1	3.0	0.2	0.0	0.0	0.3	19	0.99
	37–55	0.7	0.7	0.9	1.2	0.1	0.0	0.0	0.1	2.8	0.97
Nigeria-19	0–25	2.4	0.6	1.0	0.9	19	4.8	0.5	4.5	12	0.52
	25–60	0.5	0.1	0.2	0.3	3.0	0.5	0.0	0.7	5.9	0.74
Scotland	0–15	20	3.1	2.7	0.9	15	0.9	25	148	151	0.95
South Africa-7	0–10	2.2	0.0	0.0	0.0	0.8	0.1	0.0	2.1	16	0.97
	10–20	0.8	0.2	0.2	0.1	0.4	0.0	0.0	1.6	23	0.99
	25–60	0.8	0.4	0.6	0.2	0.3	0.0	0.0	0.4	4.6	0.95
Spain-7	0–2	4.0	0.4	0.4	0.0	2.7	0.2	4.8	28	21	0.95
	2–15	0.8	0.2	0.3	0.0	1.1	0.1	0.5	4.5	4.3	0.90
Sweden-4	0–15	12	1.0	1.2	0.9	9.5	0.9	3.3	5.8	2.5	0.55
	17–35	0.7	0.2	0.5	0.2	1.5	0.2	0.9	2.0	1.7	0.75
Texel -1	0–15	4.0	5.0	1.0	0.0	0.8	0.0	4.5	48	75	0.99
Texel-2	0–15	4.2	2.2	1.0	0.0	0.3	0.0	10	139	249	1.00
Zaire-1	0–15	7.0	1.9	2.6	1.7	8.4	2.2	0.7	10	112	0.95
	15–32	2.5	0.9	1.3	1.2	1.8	0.3	0.0	1.1	21	0.94
	40–70	1.6	0.9	1.4	1.7	0.6	0.1	0.0	0.3	7.7	0.94
	80–110	1.6	1.4	2.1	2.8	1.0	0.1	0.0	0.2	4.3	0.85
	120–150	3.5	1.8	2.9	2.9	1.7	0.1	0.0	0.4	5.3	0.81
Zaire-2	0–8	1.7	0.6	0.6	0.4	1.1	0.1	0.0	2.7	113	0.99
	10–30	0.6	0.3	0.4	0.4	0.4	0.1	0.0	0.7	16	0.98

See Appendix for structures.

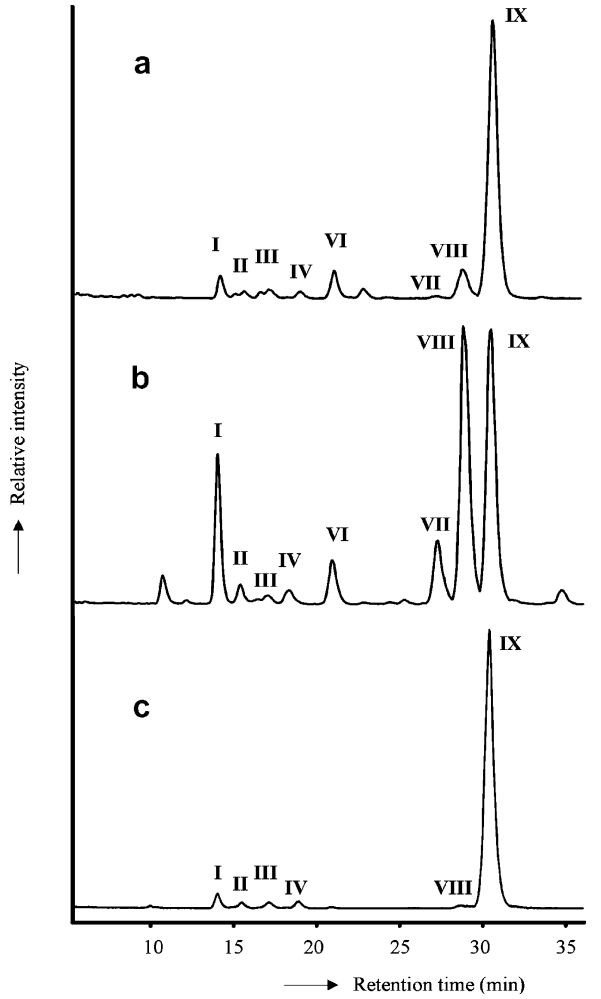

Fig. 1. HPLC–MS base peak chromatograms for three soils (top sections) from: (a) Gabon-1, (b) France-15 and (c) Nigeria-15, showing relative GDGT distribution.

concentrations of the cyclopentane-containing isoprenoid GDGTs (**II**–**IV**) are summed and plotted in one graph. These concentrations are rather invariable in the Zairian and French profile, although a slight increase (Zaire; Fig. 2a) or decrease (France; Fig. 2b) is noticeable. In the Nigerian profile (Fig. 2c) a clear maximum for GDGTs **II**–**IV** is present in the second depth interval, which resembles that of GDGT **I**.

4. Discussion

4.1. GDGTs in soils

4.1.1. Branched GDGTs *VII*–*IX*

These were found in every sample and appear to be the most abundant type of GDGT in soil (Fig. 1, Table 2). Their concentration is highest in the upper soil horizon and decreases rapidly with depth (Fig. 2a–c). This is in contrast with peat bogs, where concentrations of branched GDGTs increase with depth and are clearly highest in the anoxic part of the profile (Fig. 2d; Weijers et al., 2006). From this depth profile in peat bogs it was suggested that the bacteria producing the branched GDGTs are anaerobic microorganisms, possibly involved in organic matter mineralization (Weijers et al., 2006). In this deeper anoxic part of the peat bogs enough substrate is available for these microbes. In the deeper soil layers this might not be the case as TOC concentrations decrease substantially with depth (Table 1), which might reduce the abundance of branched GDGT-producing bacteria. The branched GDGT-producing bacteria in the upper soil layers and in the upper zone of peat bogs might be facultative aerobes, but more likely thrive in anoxic micro-habitats in (water filled) pores in the soils. A similar phenomenon has been observed for anaerobic methanogenic Euryarchaeaota in a tundra soil. These Archaea became more abundant a few days after anoxic incubation of the soil, improving the growth conditions (West and Schmidt, 2002). Concentrations of branched GDGTs normalized to TOC in soils are only slightly lower than those in the upper part (acrotelm) of peat bogs (Table 3), indicating that in the upper, better aerated part of soils and peat bogs the availability of anoxic micro-habitats rather than available substrate might be the primary limiting factor for the abundance of branched GDGT-producing bacteria.

4.1.2. Crenarchaeol *VI*

GDGT **VI** (crenarchaeol), a specific biomarker for the group 1.1 Crenarchaeota (Sinninghe Damsté et al., 2002c), is nearly always detected in marine and often in lacustrine sediments (Schouten et al., 2000; Powers et al., 2004) and has only recently been shown to occur in peat bogs (Weijers et al., 2004) and even in some hot springs (Pearson et al., 2004). Although molecular ecological studies have revealed the presence of such non-thermophilic Crenarchaeota in soils (e.g. Bintrim et al., 1997; Jurgens et al., 1997; Buckley et al., 1998; Pesaro and Widmer, 2002; Ochsenreiter et al., 2003), their unique GDGT membrane lipid, crenarchaeol (**VI**), has never been reported in soil. This can partly be attributed to the amplification technique used in molecular biology studies, enabling detection of

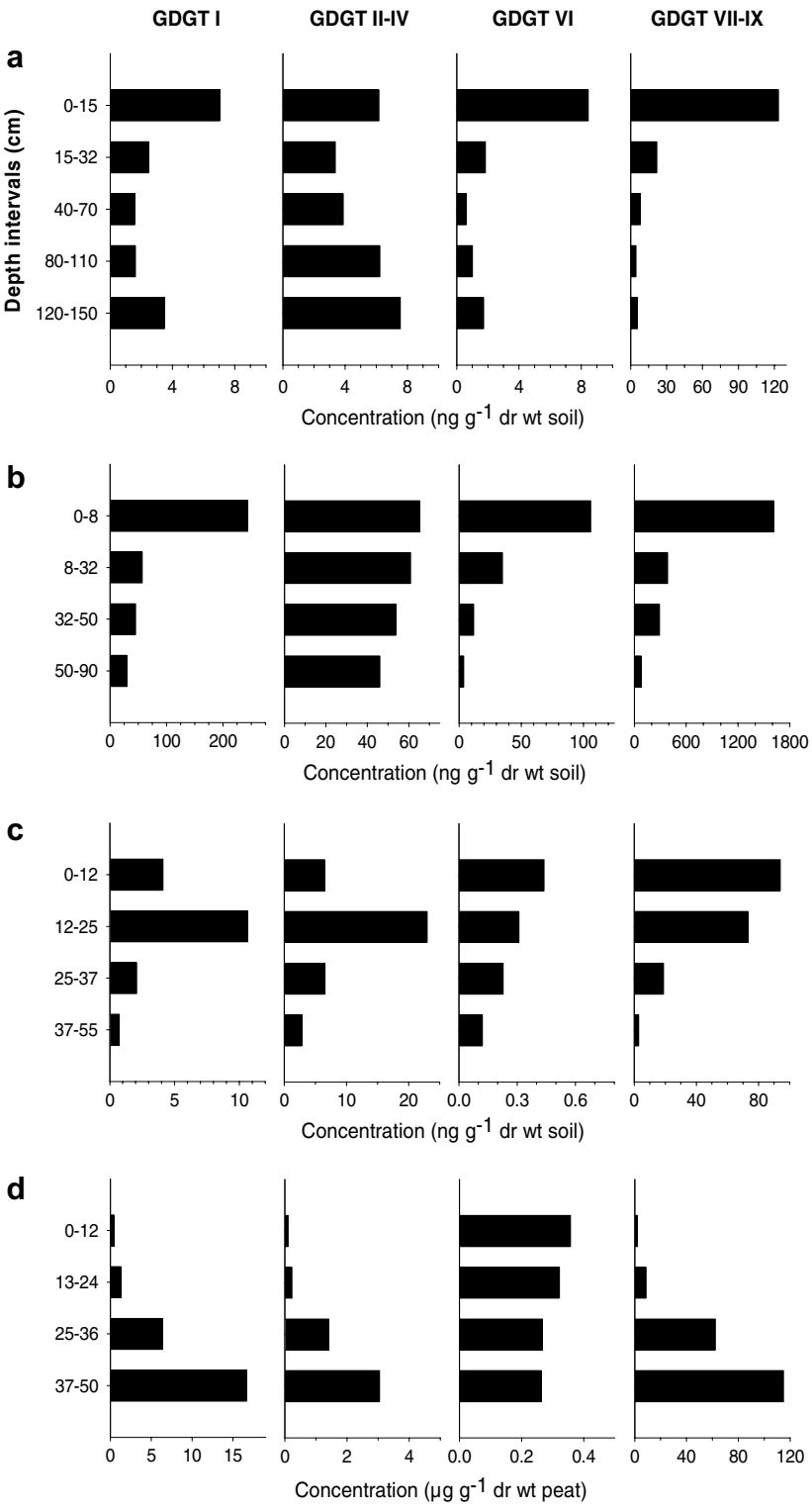

Fig. 2. Depth profiles of different GDGTs in soils from: (a) Zaire-1, (b) France-15 and (c) Nigeria-15 and of (d) the Saxnäs Mosse peat bog, Sweden (data from Weijers et al., 2004, 2006). Note the different unit used for *x*-axis in profile (d).

Table 3
Comparison of GDGT concentrations (μg g^{-1} TOC) in different environments[a]

	GDGT I	GDGT II–IV	GDGT VI	GDGT VII–IX	
Soils					
Top layers	0.4 (±0.9)	0.2 (±0.3)	0.5 (±0.7)	4.5 (±6.1)	This study
All depth intervals	0.4 (±0.8)	0.4 (±0.6)	0.3 (±0.5)	4.0 (±5.1)	
Peat bogs					
SNM acrotelm	4 (±3)	0.5 (±0.5)	0.8 (±0.8)	13 (±12)	Weijers et al. (2006)
BFM acrotelm	n.d.[b]	n.d.	n.d.	11 (±13)	
SNM catotelm	32 (±25)	8 (±5)	0.8 (±0.5)	240 (±160)	
BFM catotelm	38 (±32)	n.d.	n.d.	180 (±60)	
Marine surf. sed.					
Congo Fan	29	16	57	20	Unpublished results
Niger Fan	98	53	162	5	

[a] For the peat bogs a TOC value of 40% was assumed for recalculating concentrations from μg g^{-1} dry weight peat to μg g^{-1} TOC. SNM, Saxnäs Mosse (Sweden); BFM, Bolton Fell Moss (Cumbria, UK); Acrotelm, zone in which the water table fluctuates; and Catotelm, permanent water saturated zone.
[b] n.d., not determined.

genes at much lower concentrations than possible from lipid analysis. Nevertheless, the presence of crenarchaeol (**VI**) in nearly all soils in the current study, covering a large geographical range, confirms that crenarchaeol, and consequently its producers, members of the group 1.1 Crenarchaeota, are more widespread than previously thought.

The abundance of crenarchaeol (**VI**) relative to the total GDGT content in soils is higher than in peat samples, but still low compared to crenarchaeol in marine sediments, where it typically comprises about 50% of all GDGTs (Table 4). Concerning the microbial population, Ochsenreiter et al. (2003) determined the abundance of Crenarchaeota in soils to be 0.5–3% relative to bacterial rDNA and Buckley et al. (1998) calculated an abundance of $1.42 \pm 0.42\%$ relative to total 16S rRNA. This is indeed much lower than in the marine water column where the abundance of pelagic Crenarchaeota can reach as much as 39% of total DNA-containing picoplankton (20% on average) (Karner et al., 2001).

Interestingly, the abundance of crenarchaeol in soils is higher with increasing pH (Fig. 3). This relationship seems to be stronger than that observed for other GDGTs (e.g. the branched GDGTs, Fig. 3), and, therefore, cannot be attributed solely to a change in the relative abundance of other GDGTs with increasing pH. Phylogenetic analysis of soil Crenarchaeota often reveals a close relationship with the marine Crenarchaeota (e.g. Jurgens et al., 1997; Ochsenreiter et al., 2003). Considering that the average oceanic pH is about 8.2, it might not be surprising that relatives living in soils also prefer alkaline conditions. This observation also fits with the results of Pearson et al. (2004), who found

Table 4
Relative abundance (%) of GDGTs in different environments

	GDGT I	GDGT II–IV	GDGT VI	GDGT VII–IX	
Soils					
Top layers	7	3	9	81	This study
All depth intervals	7	9	7	77	
Saxnäs Mosse Bog					
Acrotelm	20	3	4	73	Weijers et al. (2004, 2006)
Catotelm	11	3	0.3	86	
Marine surf. sed.					
North Sea	47	6	47	Not produced in situ	Herfort et al. (2006)
Congo Fan	30	16	55		Unpublished results
Niger Fan	32	16	52		Unpublished results

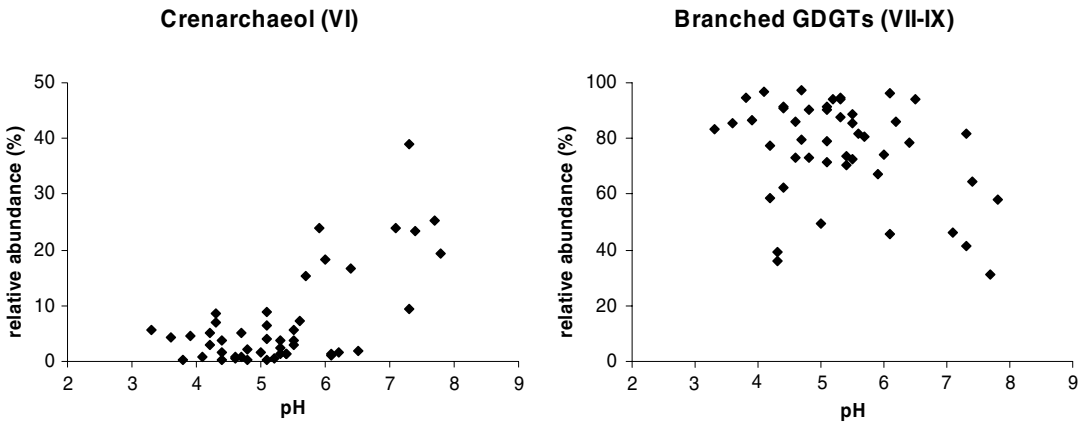

Fig. 3. Plots showing relationship between pH of soils and relative abundance (to total GDGT content) of crenarchaeol (**VI**; left) and of branched GDGTs (**VII–IX**; right).

higher crenarchaeol abundances in neutral to alkaline hot water springs. The 16S rRNA gene sequences from those springs were, however, related to thermophilic Crenarchaeota and no sequences related to the crenarchaeol-producing, non-thermophilic group 1.1 Crenarchaeota were found. This paradox might now be explained by our results, suggesting that the crenarchaeol detected in these hot springs might have been derived from surrounding alkaline soils instead of from in situ production.

*4.1.3. Isoprenoid GDGTs **I–IV***

GDGT **I** is present in virtually all the soils. Its abundance relative to total GDGT content is, however, lower in soils (7%) than in peat bogs (11–20%) and about equal to that of GDGT **VI** (crenarchaeol) in soils (7–9%; Table 4). This difference between soils and peat bogs might be the result of a much lower contribution of methanogenic Euryarchaeota, which can contain GDGT **I** in high amounts (Koga et al., 1993), to the total microbial community in soils. In contrast to peat bogs, where methanogens (Euryarchaeota) can comprise up to 36% of the total microbial community (Kotsyurbenko et al., 2004), little data are available on the abundance of Euryarchaeota in soils. Molecular ecological studies of soils have mainly revealed sequences belonging to the non-thermophilic Crenarchaeota and have rarely revealed clones belonging to the Euryarchaeota (e.g. Bintrim et al., 1997; Jurgens et al., 1997; Ochsenreiter et al., 2003). In British upland grassland soils, for example, archaeal communities seem to be dominated by non-thermophilic Crenarchaeota and sequences of putative methanogenic Euryarchaeota could only be retrieved after enrichment experiments in an anaerobic microcosm (Nicol et al., 2003a,b). A similar enrichment study was performed by West and Schmidt (2002), which showed the presumably initial presence of small amounts of (methanogenic) Euryarchaeota in anaerobic micro-sites in soils. Pesaro and Widmer (2002) could also detect only a few euryarchaeal sequences in a Swedish forest soil, which were related to Thermoplasmales. The thermophilic *Thermoplasmales acidophilicum* is also known to produce GDGT **I** (Langworthy, 1977). The GDGT membrane lipid data for the soils reported here are thus in good agreement with these literature data; GDGT **I** is probably produced mainly by non-thermophilic soil Crenarchaeota and only to a lesser extent by Euryarchaeota. Both non-thermophilic Crenarchaeota and methanogenic Euryarchaeota also produce small amounts of cyclopentane-containing GDGTs, explaining the occurrence of GDGTs **II–IV** in soils. The fact that the GDGTs **II–IV** do not always follow the distribution pattern of either GDGT **I** or **VI** (e.g. Fig. 2) could be a result of this dual source. In addition, Pancost et al. (2000) found, after ether bond cleavage of the GDGTs in two peat bogs by HI treatment, different $\delta^{13}C$ values for the acyclic and the monocyclic biphytanes. This suggests that within the methanogenic Euryarchaeota in peat bogs different groups also occur, utilizing different metabolic pathways, which produce cyclopentane-containing GDGTs (**II–IV**) in different proportions relative to GDGT **I**.

4.2. Geochemical implications

4.2.1. BIT index

Although branched GDGTs are not present in such high concentrations as in peat bogs, soils are widespread and represent such a substantial part of the terrestrial realm, such that they likely form the major source of the branched GDGTs in marine and lacustrine environments. This strongly supports the use of the BIT index as a proxy for TOM input into the marine environment (Hopmans et al., 2004). The terrestrial end member value of the BIT index is assumed to be close to 1, meaning that no, or only insignificant amounts of, crenarchaeol will be initially present in TOM. In that respect, it is important to note that, albeit in relatively low concentration, GDGT **VI** (crenarchaeol) is unambiguously present in soils (Table 3) and that this could possibly complicate the use of the BIT index here. However, the BIT index of the vast majority of soils is > 0.90, although a few soils do show substantially lower values (Fig. 4). Of these, the lowest values are found in the uppermost soil layers and are possibly the result of good aeration of these layers. In soils, the relative abundance of non-thermophilic Crenarchaeota, which are suggested to be able to thrive in both oxic and anoxic environments (Sinninghe Damsté et al., 2002b; Weijers et al., 2004), is likely higher in the top layers, because of a lower abundance of branched GDGT-producing bacteria, which seem to be obligate anaerobes (Weijers et al., 2006). Indeed, BIT indices for the subsequent lower depth interval are much higher again. The average BIT index of all the soils here is 0.91, which is still substantially higher than BIT indices of open marine settings, which usually are close to 0 (Hopmans et al., 2004). Therefore, it can be concluded that, despite the presence of GDGT **VI** (crenarchaeol) in soils, the BIT index as proxy for TOM input in the marine environment is still applicable, although with a somewhat narrower range than previously suggested.

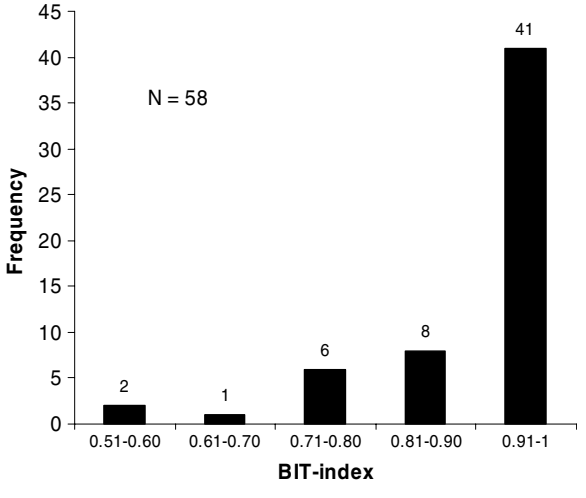

Fig. 4. Frequency histogram of different BIT ranges showing that the majority of the soils from this study has a BIT index >0.90.

4.2.2. TEX_{86} SST proxy

The TEX_{86} proxy is based on the relative distribution of the cyclopentane-containing isoprenoid GDGTs (**II**–**IV**) and the regio-isomer of crenarchaeol (GDGT **VI′**) in marine and lacustrine pelagic Crenarchaeota. The unambiguous presence of the cyclopentane-containing GDGTs (**II**–**IV**) and GDGT **VI′** in peat bogs and soils shown here and in previous studies (Gattinger et al., 2003; Weijers et al., 2004), suggests that when those GDGTs are fluvially transported to marine or lacustrine environments, they could bias the TEX_{86} signal. It is important to consider, however, that the absolute concentrations of GDGTs **II**–**IV** and **VI′** in peat bogs and soils are about one to two orders of magnitude lower than those in marine sediments (Table 3) and that consequently a substantial input of TOM is required to alter the marine TEX_{86} value. However, especially near the outflow of large rivers, a bias in TEX_{86} might occur and could alter the reconstructed SSTs. This may also hold for reconstructing lake surface temperatures as proposed by Powers et al. (2004), because crenarchaeol abundances in lakes seems to be lower than in the marine environment (Powers et al., unpublished results) and lakes usually receive a relatively large amount of TOM.

An approximation for determining the relative amount of TOM input, and therefore a possible bias in the TEX_{86} signal, can be derived from the BIT index. As mentioned above, the BIT index of soils is in most cases > 0.90. In general, it can be assumed that if BIT indices of marine or lacustrine sediments are low, i.e. a relatively low terrestrial GDGT input compared to marine GDGT production, the TEX_{86} proxy can safely be used, as the vast majority of the isoprenoid GDGTs used for calculating this proxy will in these cases be of marine or lacustrine origin. If BIT indices in these sediments are higher, however, the TEX_{86} proxy might be altered to a certain degree by the input of terrestrially derived GDGTs.

To illustrate the potential effect of the terrestrially derived GDGTs on the TEX_{86} proxy, a simple two end member mixing model was used for the equatorial East Atlantic region. As terrestrial end member, a GDGT lipid mixture was taken representing the average GDGT lipid distribution of the African soils, with a BIT index of 0.92 and a TEX_{86} derived 'temperature' of 32.2 °C (Fig. 5a). As marine end member, a GDGT lipid mixture representing the GDGT distribution in a marine sediment sample from core GeoB-4901 from the Niger deep sea fan was taken, with a BIT index of only 0.03 and a TEX_{86} temperature of 22.3 °C (Fig. 5b). For different mixing ratios of these end members, i.e. a virtual transect from the open marine setting into the river mouth, the BIT index and TEX_{86} SST were calculated. The temperature deviation from the original TEX_{86} derived temperature of the marine GDGT lipid mixture is plotted together with the BIT index (Fig. 5c). The curves clearly show that, at higher BIT indices, the temperature deviation increases substantially in a non-linear way. In this particular example, a temperature deviation of +1 °C, the analytical error in the TEX_{86} proxy, is reached at a BIT index of 0.2–0.3, and a deviation of >2 °C is reached at a

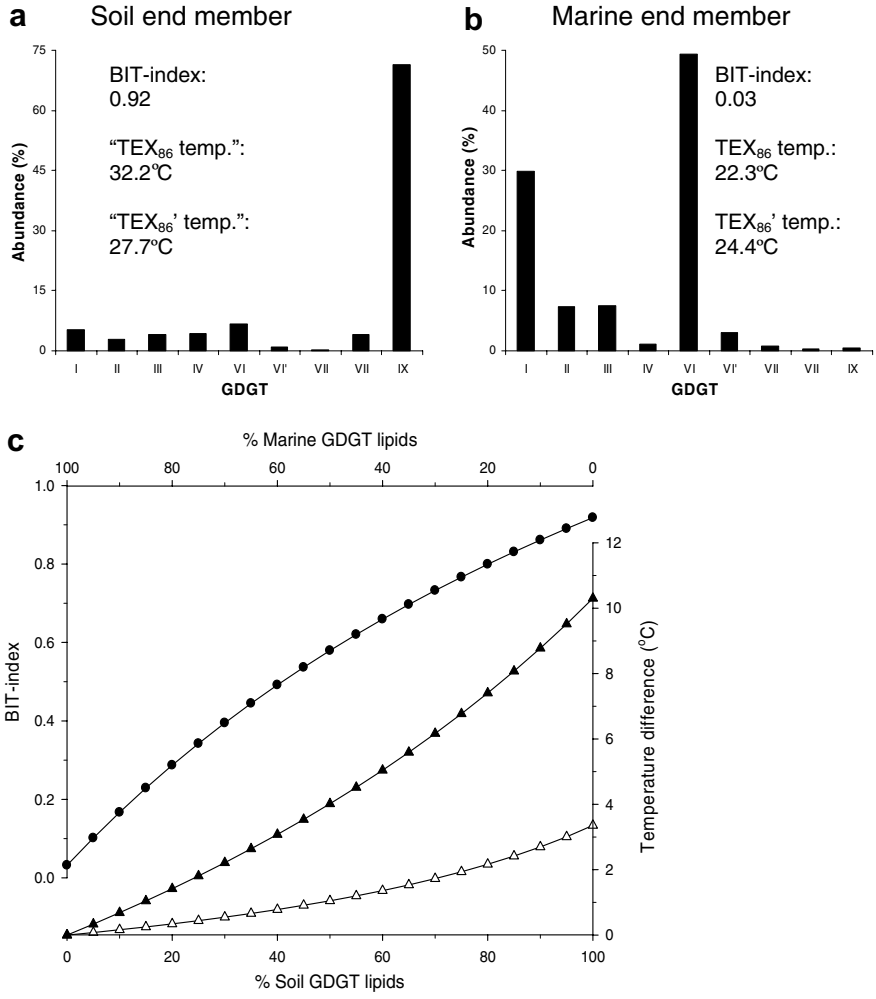

Fig. 5. Hypothetical binary mixing model for equatorial Atlantic region composed of (a) an end member representing average GDGT distribution in the African soils, and (b) an end member representing the GDGT distribution in a marine sediment sample from core GeoB 4901 (Niger deep sea fan). Graph (c) shows with different mixing ratios the positive temperature difference from the original marine end member value according to the TEX_{86} proxy (black triangles) and the TEX'_{86} proxy (white triangles) with the accompanying BIT indices (black dots).

BIT index of 0.4. These seem to be large deviations, but it is important to recall that reaching a BIT index of 0.4 represents a considerable TOM input, as concentrations of GDGTs in soils are much lower than in the marine environment (Table 3). In this particular example, with a BIT value of 0.4, >30% of the GDGTs is derived from soils and, consequently, >95% of the TOC is derived from TOM. Furthermore, the soil GDGT mixture taken here as a terrestrial end member will often be mixed with GDGTs derived from Crenarchaeota living in lakes and rivers, which will also be fluvially transported to the marine environment. The GDGT mixture in those lake and river Crenarchaeota is not altered by methanogen-derived GDGTs and, therefore, contains a lower TEX_{86} value, which will consequently diminish the difference in GDGT distribution between the soil and marine environment.

Recently, an alternative TEX'_{86} proxy was applied by Sluijs et al. (2006) in a sediment core from the Arctic, covering the Palaeocene-Eocene thermal maximum interval, where exceptionally high levels of GDGT **IV** were found at intervals with a high BIT index (0.5–0.7). The high abundance of GDGT **IV** was attributed to high levels of terrestrial input and therefore GDGT **IV** was left out of the TEX_{86} formula. Indeed, our results confirm that in soils the relative abundance of GDGT **IV** is often higher than in marine sediments (e.g. Fig. 5a and b). When the TEX'_{86} was calculated for our hypothetical binary mixing model, it indeed resulted in a much reduced temperature bias, and a +1 °C deviation is only reached at a BIT index of about 0.6 (Fig. 5c). It should be noted, however, that this reduced bias will depend on the relative abundance of GDGT **IV** in soils, which can vary to a large degree. This variation will partly be due to differences in the abundance of different groups of methanogenic Euryarchaeota, as discussed above.

Regardless of using either the TEX_{86} or the TEX'_{86} proxy, the temperature deviation in the binary mixing model strongly depends on the TEX_{86} value of the end members. The 'TEX_{86} signal' for soils might differ with region, as will the TEX_{86} signal of the marine end member. The largest temperature deviations are likely to occur at places with cool ocean waters as soils generally contain a relatively warm TEX_{86} signal. If TEX_{86} SSTs in such cases are determined over a time interval in which BIT indices remain constant, relative changes in TEX_{86} SSTs can still, however, be used, even though the absolute SST is biased. Overall, care should be taken in interpreting TEX_{86}-derived absolute SSTs and determination of the terrestrial GDGT input with the BIT index is required in all lakes and near coastal areas, especially near large river outflows.

5. Conclusions

Branched GDGTs (**VII–IX**) are abundant in all the soils investigated, confirming their widespread occurrence in the terrestrial environment. This shows that the BIT index is a powerful tool for tracing relative terrestrial organic matter input in the marine environment. Detection of GDGT **VI** (crenarchaeol) in virtually all the samples, a specific biomarker for the non-thermophilic (group 1.1) Crenarchaeota, confirms molecular ecological studies that have shown the occurrence of these organisms in soils. As the abundance of crenarchaeol (**VI**) in soils relative to the branched GDGTs (**VII–IX**) is very low compared to the marine environment, its presence does not have a large influence on the BIT index used in marine settings. Cyclopentane-containing isoprenoid GDGTs (**II–IV**) were, although in low concentration, also detected in soils. They could, when fluvially transported to the marine environment, bias TEX_{86}-derived SSTs in near coastal areas and lakes, especially near large river outflows. At these places, therefore, quantification of the relative TOM input with the BIT index is required to determine a possible bias in the TEX_{86} proxy.

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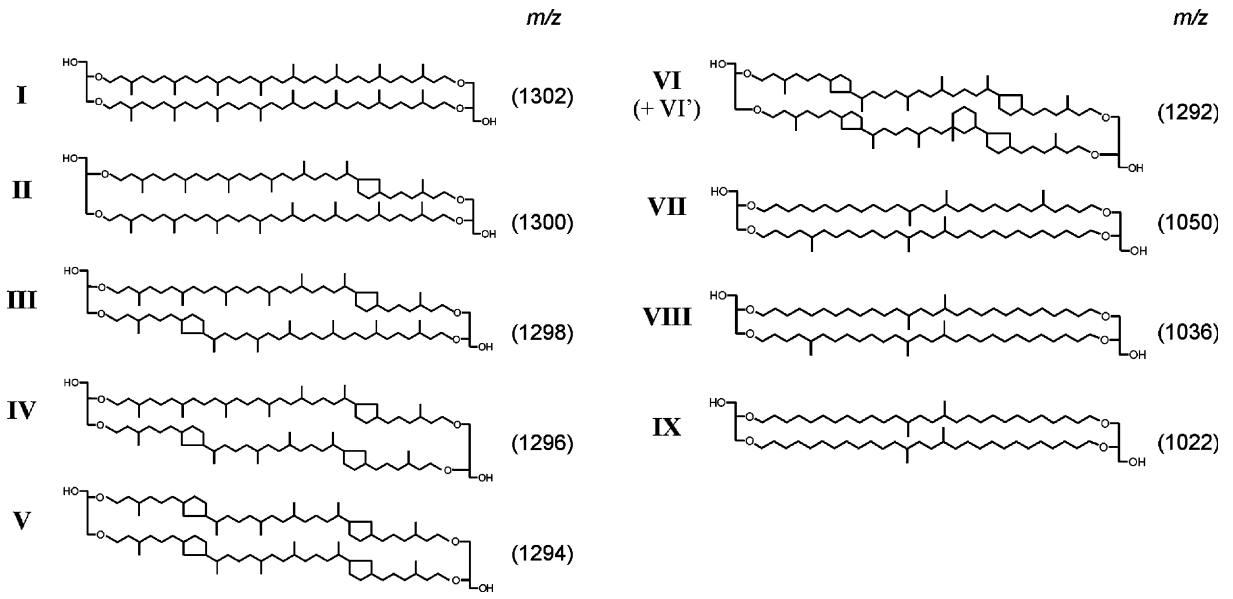
Appendix



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