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Bacteriohopanepolyols in tropical soils and sediments in the Congo

River catchment area

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ABSTRACT

The Congo River basin drains the second largest area of tropical rainforest in the world, including a large proportion of pristine wetlands. We present the full bacteriohopanepolyol

(BHP) inventory from a suite of tropical soils and, by comparison with other published data, propose some initial ideas on BHP distribution controls. Strong taxonomic controls on BHP production are evident in wetland sediments. 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol) and 35-aminobacteriohopane-30,31,32,33,34-pentol (aminopentol) were dominant within the BHP suite, indicating aerobic methanotrophy. A small range and low mean relative abundance of 30-(5'-adenosyl)hopane (adenosylhopane) and related compounds collectively termed "soil marker" BHPs was observed in Congo soils (mean 17%, range 7.9-36% of total BHPs, n = 22) compared with literature data from temperate surface soils and Arctic surface soils (mean 36%, range 0-66% of total BHPs, n = 28) suggesting a greater rate of conversion of these BHP precursors to other structures.

Key words

Bacteriohopanepolyols, adenosylhopane, tropical soil, methanotrophic bacteria, Congo, wetland

1. Introduction

Bacteriohopanepolyols (BHPs) are highly functionalised pentacyclic triterpenoids produced by many aerobic bacteria, as well as a number of obligate and facultative anaerobic bacteria (e.g. Rohmer et al., 1984; Sinninghe Damsté et al., 2004; Talbot et al., 2008; Eickhoff et al., 2013). Only bacteria containing the gene encoding for squalene hopane cyclase (*sqhC*;

Ochs et al., 1992) are able to biosynthesise hopanoids. Biosynthesis of BHPs is believed to be limited to < 10% of all bacterial species in most communities (Pearson et al., 2007). The initial step in BHP synthesis is the cyclisation of squalene (controlled via the *sqhC* gene) with the addition of the hopanoid side chain (via the *hpnH* gene) and leading to the production of 30-(5'-adenosyl)hopane (adenosylhopane; **1a**; Fig. 1; Bradley et al., 2010). It is believed that all hopanoid producing bacteria synthesise adenosylhopane as a BHP precursor compound, however, few hopanoid producers have been observed accumulating adenosylhopane and only one species has been found to contain the related compound adenosylhopane type 2 (**1c**) (e.g. Talbot et al., 2007 and references therein; van Winden et al., 2012a). All species in which adenosylhopane has been identified were also found to contain a range of other BHPs including bacteriohopane-32,33,34,35-tetrol (BHT), 35-aminobacteriohopane-32,33,34-triol (aminotriol) or both (Talbot et al., 2007a, 2008; van Winden et al., 2012b). These and other BHPs are formed following cleavage of the adenine moiety (Bradley et al., 2010; Liu et al., 2014), however, it is currently unknown, why accumulation of adenosylhopane only occurs in terrestrial systems (soils in particular), and not in marine sediments. This suggests that the function of adenosylhopane is not restricted to that of a biosynthetic precursor or it would likely accumulate in all settings.

While many BHPs have been identified as having multiple bacterial sources, for example BHT (**1b**; Fig 1; e.g. Talbot et al., 2008 and references therein), some have only a few sources and can be linked to specific biogeochemical processes. Adenosylhopane (**1a**) and related compounds, including C-2 methylated homologues (**2a**, **1c**, **2c**, **1d** and **2d**), have been suggested to be biomarkers for soil organic carbon (OC) transport (Cooke et al., 2008b, 2009; Zhu et al., 2011; Doğrul Selver et al., 2012, 2015). Another group of diagnostic markers are those produced by aerobic methane oxidising bacteria (methanotrophs) including 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol; **1e**); 35-aminobacteriohopane-30,31,32,33,34-pentol (aminopentol; **1f**), unsaturated aminopentol

(**4/5f**) and aminopentol isomer (**1f'**; e.g. Talbot and Farrimond, 2007; Zhu et al., 2010; van Winden et al., 2012b; Berndmeyer et al., 2013; Talbot et al., 2014).

BHP signatures in the geological record are thought to reflect changes in microbial communities at the time of deposition, with multiple factors controlling their distribution. For example, Wagner et al. (2014) suggests aminopentol in sediments dating back 30 Ka from the Amazon fan, are derived from the Amazon catchment with fluctuations in concentration reflecting persistent export of biomarkers from wetlands followed by reworking of sediments within the marine environment. An investigation of suspended particulate matter (SPM) along a tropical river-ocean water column transect also suggested that terrigenous organic matter (OM) exported to marine sediments could provide a significant contribution to the marine sedimentary hopanoid inventory (Sáenz et al., 2011). Therefore in coastal marine environments well constrained modern terrestrial BHP end members are required to facilitate reliable interpretation of sedimentary BHP profiles.

Studies of soil BHP distributions have focussed mainly on Northern Hemisphere sites (Cooke et al., 2008a; Xu et al., 2009; Cooke, 2010; Rethemeyer et al., 2010; Kim et al., 2011) and found high concentrations of BHT (**1b**), aminotriol (**1g**) and bacteriohopanetetrol carbopseudopentose ether (BHT cyclitol ether; **1h**), together with adenosylhopane (**1a**) and some or all of the related compounds **2a**, **1c**, **2c**, **1d**, **2d**. In comparison, few studies detail the distribution of BHPs in modern tropical soils (Pearson et al., 2009; Wagner et al., 2014). Soils generally contain higher BHP concentration and greater structural diversity than lacustrine and marine sediments (Cooke et al., 2008b; Talbot and Farrimond, 2007; Coolen et al., 2008; Blumenberg et al., 2010; Zhu et al., 2011), with the exception of deep sea-fan sediments with very high terrestrial input (Handley et al., 2010; Wagner et al., 2014). For example Cooke et al., (2008a) reported high structural diversity and concentration of hopanoids in soils (up to 20 BHPs identified in two of four surface soils from the Northern UK), and Zhu et al. (2011) identified up to 20 BHP compounds in a soil from the Yangtze

River catchment. However, a recent study of two surface soils and three surface wetland sediments from the Amazon found the highest BHP concentrations and greatest structural diversity within wetland sediments (18 BHPs in sediments vs. 13 in the soils; Wagner et al., 2014), suggesting wetlands as possibly a significant source of BHPs to shelf and fan systems. As tropical wetlands and soils are largely understudied, large uncertainty in BHP end members likely exists.

The Congo basin consists of a large contrast in tropical environments with humid tropical rainforest, extensive wetlands and savannah environments (Spencer et al., 2012, 2014). Previous work on sediments from the Congo fan suggests terrigenous OC input as an important source of BHPs in these coastal marine sediments (Cooke et al., 2008b; Talbot et al., 2014). In this study we have determined the BHP inventory of 22 soils and 6 wetland sediments (Malebo pool) from the Congo hinterland and 1 estuarine sediment from the mouth of the Congo River (Fig. 2). We discuss the application of BHPs as biomarkers for soil OC transport and biogeochemical cycling and review the significance of the distributions in the context of reported soil BHP data.

2. Material and methods

2.1. Site location and sample description

The sediment from the estuary of the Congo River ('Anker 24') was taken as a grab sample (Eisma et al., 1978) and stored as dried sediment before analysis. Additional lipid data have been published (Schefuß et al., 2004).

Details of the soil and Malebo pool sample collection have been reported (Talbot et al., 2014). Briefly, soil samples were collected from 22 sites spanning a wide range of land cover types, ranging from scrub savannah and grasslands, secondary forest and pristine tropical mixed forest, to seasonally flooded and swamp forest environments within the Congo Basin (Fig. 2). Surface soil samples (0-5 cm) were collected in November 2010 and August 2011.

Sites were ca. 5-30 m from nearby streams and rivers. Samples were wrapped in clean Al foil, shipped to Newcastle University (UK) within three weeks of collection and, stored frozen on arrival and freeze dried and ground prior to lipid extraction.

Malebo Pool floodplain wetland sediments were collected along a transect at three sites encompassing permanently flooded sediment, sediment inundated during high discharge months only and sediment from above the seasonal high water point (Fig. 2). At each of the sites sediment was collected at two distinct depths (0-5 cm and 5-15 cm), i.e. a surface and sub-surface sample. Samples were immediately frozen and shipped to Newcastle University (U.K.).

2.2. *pH*

The pH was measured following the standard method described in BS ISO 10390 (2005). Briefly 5 ml soil were shaken with 25 ml water for 1 h and the resulting soil-water suspension left to equilibrate for 1 to 3 h. The pH of the suspension was measured using a pH electrode (VWR 662-1761; combination double junction with BNC connector ATC temperature probe, Dutscher Scientific, part no. 027-017) and meter (Jenway 3020, serial no. 2539), calibrated using standard buffer solutions of pH 4 and 7.

2.3. *Total OC (TOC)*

TOC (%) of the soils and Malebo Pool samples was measured at Newcastle University. Approximately 0.1 g of sample was treated with 4 mol/L HCl (60-70 °C) for removal of inorganic carbon. Following which, HCl was allowed to drain from each sample. Deionised water was added to each sample to neutralise the acid and allowed to drain. The samples were then dried in an oven at 65 °C for between 16 and 24 hours. TOC was measured using a LECO CS244 Carbon/Sulfur Analyser. Precision based on repeat sample analysis was 4.5 % (relative standard deviation). Accuracy based on repeated measurements of a standard reference material (Chinese stream sediment, NCS DC 73307; LGC, Teddington, UK) was within the permissible ± 0.05 % TOC. An instrument calibration standard (Carbon in

steel, part no 501-506, Leco) was analysed and was found to be within the nominal 0.8% permissible range.

2.4. Lipid extraction

Freeze-dried samples (ca. 3 g) were extracted using a modified Bligh and Dyer method as described by Cooke et al. (2008a). Material was extracted in a Teflon centrifuge tube with addition of a monophasic solution of water/MeOH/CHCl₃ (4:10:5, v/v). The mixture was sonicated at 40 °C for 1 h followed by shaking at room temperature for 2-4 h. The mixture was then centrifuged at 12,000 rpm for 15 min and the supernatant transferred to a second centrifuge tube. This process was repeated 3 times. The decanted supernatant was phase separated using CHCl₃ (5 ml) and water (5 ml). The tubes containing the supernatants were centrifuged for 5 min to complete the separation of the organic (CHCl₃) and MeOH/water phases. The combined organic (CHCl₃) fraction was transferred to a round bottomed flask and rotary evaporated to near dryness. The extract was transferred to a glass vial using a solution of warm (ca. 50°C) CHCl₃/MeOH (2:1, v/v). The total lipid extract (TLE) was evaporated to dryness under a stream of N₂. A 5 α -pregnane-3 β ,20 β -diol internal standard was added (0.236 μ g/ μ l) and the TLE split into 3 equal aliquots following dilution with 5 ml CHCl₃/MeOH (2:1, v/v; heated at 50 °C for 10 min).

2.5. BHP analysis

One third of the TLE was used for BHP analysis: the aliquot was evaporated to dryness under N₂ and acetylated by adding Ac₂O (1 ml) and pyridine (1 ml). This aliquot was then heated for 1 h (50 °C) and left at room temperature overnight. The Ac₂O and pyridine were removed under a stream of N₂ and the resulting acetylated extract was dissolved in 1 ml MeOH/propan-2-ol (3:2, v/v).

BHP analysis was performed by reversed phase high performance liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry (HPLC-APCI-MSⁿ) using a ThermoFinnigan surveyor HPLC system fitted with a Phenomenex Gemini C₁₈ column (150

mm; 3.0 mm i.d.; 5 μ m particle size) and a security guard column cartridge of the same material coupled to a Finnigan LCQ ion-trap mass spectrometer equipped with an APCI source operated in positive ion mode. Chromatographic separation was accomplished at 30 °C at 0.5 ml/min with the following solvent gradient: 90% MeOH, 10% H₂O (0 min); 59% MeOH, 1% H₂O, 40% propan-2-ol (at 25 min); isocratic to 45 min returning to the starting conditions in 5 min and stabilising for 10 min. APCI was achieved at 155 °C capillary temperature and 490 °C APCI vaporiser temperature with a corona discharge current of 8 μ A, and sheath and auxiliary gas flow of 40 and 10, respectively (arbitrary units). MSⁿ analysis was carried out in data-dependent mode with three scan events: SCAN 1: full spectrum, m/z 300–1300; SCAN 2: data-dependent MS² spectrum of most intense ion from SCAN 1; SCAN 3: data-dependent MS³ spectrum of most intense ion from SCAN 2. Detection was achieved at an isolation width of m/z 5.0 and fragmentation with normalised collision dissociation energy of 35% and an activation Q value (parameter determining the m/z range of the observed fragment ions) of 0.15. Semi-quantitative estimation of BHP concentration was achieved employing the characteristic base peak ion areas of individual BHPs in mass chromatograms (from SCAN 1) relative to the m/z 345 mass chromatogram base peak area of the acetylated 5 α -pregnane-3 β ,20 β -diol internal standard. Averaged relative response factors relative to the internal standard, determined from a suite of acetylated BHP standards, were used to adjust the BHP peak areas (see van Winden et al., 2012b). Typical error in absolute quantification was $\pm$ 20%, based on selected replicate analyses and BHP standards of known concentration (Cooke, 2010; van Winden et al., 2012b).

2.6. Compound classification and statistics

The abbreviated names of the compounds, characteristic base peak ions (m/z) and structure numbers are given in Table 1. The term tetrafunctionalised compounds refers to BHPs with four functional groups at the C-32, C-33, C-34 and C-35 positions (Fig. 1).

204 Pentafunctionalised compounds have an additional fifth functional group at C-31 and
 205 hexafunctionalised compounds have 2 additional functional groups at C-30 and C-31.

206 BHPs diagnostic for soil OC input (hereafter “soil marker BHPs”) include adenosylhopane
 207 (**1a**), C-2 methylated adenosylhopane (**2a**), adenosylhopane type 2 (**1c**) C-2 methylated
 208 adenosylhopane type 2 (**2c**), adenosylhopane type 3 (**1d**) and its C-2 methylated homologue
 209 (**2d**). The structure of the terminal functional groups in adenosylhopane type 2 and type 3
 210 remain to be elucidated, so assignment of these compounds is based on retention time and
 211 comparison of APCI mass spectra with published data (Cooke et al., 2008a; Rethemeyer et
 212 al., 2010).

213 The R_{soil} index (as defined by Zhu et al., 2011) was calculated according to the relative
 214 concentrations of BHT (**1b**) and all soil marker BHPs. The R'_{soil} index was later proposed as
 215 an alternative index excluding methylated homologues for settings where the C-2 methylated
 216 soil marker BHPs were infrequently/intermittently present (Doğrul Selver et al., 2012) and is
 217 calculated according to the relative concentrations of BHT (**1b**) and adenosylhopane (**1a**),
 218 adenosylhopane type 2 (**1c**) and adenosylhopane type 3 (**1d**).

$$219 \quad R_{\text{soil}} \text{ index} = (\mathbf{1a} + \mathbf{2a} + \mathbf{1c} + \mathbf{2c} + \mathbf{1d} + \mathbf{2d}) / (\mathbf{1a} + \mathbf{2a} + \mathbf{1c} + \mathbf{2c} + \mathbf{1d} + \mathbf{2d} + \mathbf{1b})$$

$$220 \quad R'_{\text{soil}} \text{ index} = (\mathbf{1a} + \mathbf{1c} + \mathbf{1d}) / (\mathbf{1a} + \mathbf{1c} + \mathbf{1d} + \mathbf{1b})$$

221 AminoBHPs include aminotriol (**1g**), unsaturated (**4/5g**) and methylated aminotriol (**2/3g**),
 222 aminotetrol (**1e**) and unsaturated aminotetrol (**4/5e**), and aminopentol (**1f**), unsaturated (**4/5f**)
 223 and aminopentol isomer (**1f'**; van Winden et al., 2012a). BHPs diagnostic for aerobic
 224 methane oxidation (hereafter referred to as “CH₄ oxidation markers”) include aminotetrol (**1e**),
 225 aminopentol (**1f**), unsaturated (**4/5f**) and aminopentol isomer (**1f'**; van Winden et al., 2012a).

226 The data were found to have a non-parametric distribution and were not mathematically
 227 transformed prior to statistical analysis. Spearmans rho (r_s) was calculated using IBM SPSS
 228 statistics version 21 software. Strong correlation between two variables would result in an r_s

value of 0.9 and above. Subsurface sediment samples (PS 5-15; RE 5-15; EF 5-15) were excluded from statistical analysis as all other samples were surface samples. The estuary sample and one surface wetland sample (RE 0-5) were also excluded from statistical analysis due to the small sample size, so pH data could not be obtained for either sample.

3. Results

3.1. TOC and soil pH

TOC and soil pH values are presented in Table 2. TOC ranged from 0.23-6.11% in the soils and 1.10-2.68 % in wetland sediment samples; pH ranged from 3.09-5.75 for soils and 4.27-4.8 for wetland sediments (not measured for recently exposed sediment 0-5 and the estuary sample due to insufficient sample material).

3.2. BHPs in Congo soils

A total of 35 BHPs were detected within 22 tropical soils from the Congo hinterland, including tetra-, penta- and hexafunctionalised compounds as well as those with a cyclised side chain (Table 3 and 4). Aminotriol (**1g**) and BHT cyclitol ether (**1h**) are the dominant compounds in most of the soil samples (36-68% of aminotriol and BHT cyclitol ether in total BHPs). C-2 and C-3 methylated BHpentol cyclitol ethers (**2i** and **3i**) and BHhexol cyclitol (**2j** and **3j**) ethers were also found in the soil samples, though present as minor components (Table 3 and 4).

Aminopentol (**1f**) was present as a minor component of the BHP suite, with a concentration ranging from 0.92-47µg/g TOC within six soils. However, aminopentol was found in high concentration (260 µg/g TOC) and high relative abundance (8.8% of total BHPs) in one outlier soil (closed evergreen lowland forest sample (CELF) C18B).

The distribution of individual soil marker BHPs varied across the 22 soils, with adenosylhopane (**1a**) consistently being the most abundant of the soil marker BHPs with a

concentration ranging from 33-800 µg/g TOC. Mosaic forest/ cropland (MF) C8B was the only soil where 'adenosylhopane type 2' (**1c**) was the most abundant soil marker BHP. C-2 methylated adenosylhopane (**2a**), 'adenosylhopane type 2' and C-2 methylated 'adenosylhopane type 2' (**2c**) were present in all the soils with 'adenosylhopane type 3' (**1d**) found in all samples except swamp bushland and grassland C38B (SB C38B), CELF C27B and Gilbertiodendron forest (GF 9-1). C-2 methylated 'adenosylhopane type 3' (**2d**) was found only intermittently (Table 3 and 4). Soil marker BHPs ranged from 10-36% within the forest soils (n=16) and 7.9-36% of total BHPs within the savannah/grassland samples (n=6). R_{soil} and R'_{soil} indices were calculated for the 22 Congo soils (see Section 2.6 for definition). R_{soil} and R'_{soil} indices ranged from 0.58-0.92 (avg. 0.77) and 0.48-0.91 (avg. 0.74) respectively (Table 2).

3.3. *BHPs in wetland sediments*

A total of 19 BHPs were found in the 6 wetland sediments. BHP concentration within the wetland samples ranged between 4300 µg/g TOC (recently exposed surface and sub-surface sample, RE 0-5 and RE 5-15) and 7500 µg/g TOC (permanently submerged sub surface sample, PS 5-15). Aminopentol (**1f**) and adenosylhopane (**1a**) were the dominant BHPs along with BHT cyclitol ether (**1h**) and aminotriol (**1g**) (Table 3). The wetland sediments also contained other CH₄ oxidation markers, including aminotetrol (**1e**), aminopentol isomer (**1f'**) and unsaturated aminopentol (**4/5f**: reported by Talbot et al., 2014; Table 5).

Concentration of soil marker BHPs ranged from 620 µg/g TOC (exposed with occasional flooding sub surface sample; EF 5-15) to 1100 µg/g TOC (PS 5-15). Relative abundance of soil marker BHPs ranged from 11-17% of total BHPs. R_{soil} index ranged from 0.61-0.66 (avg. 0.63; Fig. 3) and the R'_{soil} index from 0.59-0.62 (avg. 0.60) (Table 2).

3.4. Estuarine sediment

The estuarine sample had low BHP diversity, with only 12 BHP compounds and a total BHP concentration of 1400 µg/g TOC (Table 5). Aminopentol and adenosylhopane were dominant (Table 5). Adenosylhopane was the only soil marker BHP, at 81 µg/g TOC (relative abundance 6% of total BHPs); R_{soil} index was 0.20.

4. Discussion

4.1. BHP distributions

The soils were dominated by non-source specific BHPs (Tables 3-5). Greater BHP diversity was found within soils vs. the wetland and estuarine samples, consistent with other studies (e.g. Pearson et al., 2009; Zhu et al., 2011). BHT cyclitol ether (**1h**) was one of the most dominant BHPs in the soils, wetlands and the estuarine sediments. Studies have shown that, within surface soils where aerobic methane oxidation (AMO; as indicated by aminopentol; **1f**) is not a dominant process, aminotriol (**1g**), BHT cyclitol ether, BHT (**1b**) and adenosylhopane (**1a**) are usually the dominant compounds (Cooke et al., 2008a; Pearson et al., 2009; Cooke, 2010; Zhu et al., 2011). Low concentrations of anhydroBHT (**1m**), ribonylhopane (**1k**) and BHT-pseudopentose (methylated, **2l** and non-methylated, **1l**) were also present in the soils (Tables 3 and 4); however, these compounds are not discussed further due to their intermittent occurrence and typically low concentration.

4.2. Soil marker BHPs

A range of soil marker BHP relative abundance was observed for soils (7.9-36% of total BHPs) and wetland sediments (11-17% of total BHPs; Fig. 4). However, the Congo soils had a low mean soil marker BHP abundance of 16% for forest soils (n=16) and 19% for savannah/field soils (n=6) compared with samples from other studies (Table 6). Surface soils from temperate regions show a wider range of soil marker BHP relative abundance (0-66%; Cooke et al., 2008; Xu et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011; Zhu et al.,

2011; $n = 28$) than that for tropical surface soils (7.9-36%; Pearson et al., 2009; Wagner et al., 2014; this study; $n = 25$) and tropical wetlands (2.6-17%; surface and subsurface samples; Wagner et al., 2014; this study; $n = 11$; Table 6). The difference could be due to local environmental parameters. For example, pH is known to affect BHP distributions (concentration normalised to TOC and relative abundance) in environmental samples (Kim et al., 2011) and in laboratory culture experiments where changes in the amount and/or type of BHPs produced are reported (Poralla et al., 1984; Welander et al., 2009; Schmerk et al., 2011). pH did not correlate with soil marker BHP concentration ($\mu\text{g/g TOC}$; $r_s -0.600$, $p = 0.002$), R_{soil} ($r_s -0.203$, $p = 0.341$) or R'_{soil} ($r_s -0.266$, $p = 0.209$). This suggests pH is not a key factor influencing soil marker BHP distributions in our samples; however, it should be noted that the soils here were from a narrower pH range (3.09-5.75) than those in the Kim et al. (2011; pH 4.6-8.9) study.

4.2.1. R_{soil} and R'_{soil}

These indices have been proposed as soil OM input proxies that use adenosylhopane and related compounds as indicators of soil OC and BHT as a pseudo marine end member as it is found in both soils and open marine sediments (Zhu et al., 2011; Doğrul Selver et al., 2012, 2015). As the relative changes in R_{soil} vs R'_{soil} are the same within the Congo soils and sediments, only R_{soil} will be discussed. There was a wide range of R_{soil} values for the Congo forest and savannah/field soils, with a smaller range for the wetland samples (Fig. 3). While there was a clear difference in R_{soil} index between the catchment and the estuary, R_{soil} did not distinguish between the catchment sub-environments (Fig. 3). Data collated from previous tropical BHP studies show R_{soil} ranging from 0.43-0.83 for tropical soils (Pearson et al., 2009; Wagner et al., 2014; $n = 3$) and 0.27-0.68 (R_{soil}) for Amazon wetlands (Wagner et al., 2014; Table 4; Fig. 3; $n = 5$). Arctic and temperate surface soils also show a wide range of R_{soil} values from 0-0.85 ($n = 28$; Cooke et al., 2008a; Xu et al., 2009; Kim et al., 2011; Rethemeyer et al., 2010; Zhu et al., 2011; Table 6; Fig. 3). These results suggest that there is no globally consistent pattern in the R_{soil} index, application of this proxy being strongly

dependent on local end members (Zhu et al., 2011). The R_{soil} index for the Congo samples correlated weakly with the concentration of BHT (R_{soil} r_s -0.616, p 0.001; $\mu\text{g/g}$ TOC) but not with total soil marker BHP concentration (R_{soil} r_s -0.092, p 0.671; $\mu\text{g/g}$ TOC).

The R_{soil} (Zhu et al., 2011; Doğrul Selver et al., 2012) and GDGT based BIT (Hopmans et al., 2004) indices have both been proposed as proxies for soil OC transport. Previous analysis of surface sediments from river-estuary-shelf/ocean transects have identified correlation between R_{soil} and BIT indices (Zhu et al., 2011; Doğrul Selver et al., 2012; 2015). Other studies, however, have not found any correlation between soil marker BHP concentrations or the R_{soil} / R'_{soil} and BIT indices (Kim et al., 2011; Wagner et al., 2014). The absence of correlation between these two proxies in terrestrial sources materials (soils, peat) is not unexpected, however, as it is well established that soil BHP distributions contain variable concentrations of the pseudo-marine endmember BHT (e.g. Cooke et al., 2008; Xu et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011) whilst most soils contain little if any crenarchaeol (the marine endmember for the BIT index; Schouten et al., 2013). This is a prominent issue with using BHT as a marine end member in soil OM proxies. Furthermore, relatively little is known about possible marine sources of BHT other than some species of sulfate reducing bacteria (e.g. Blumenberg et al., 2006). Lack of correlation between these two proxies in certain environments could be due to (1) terrestrial end member biomarkers synthesised by microbial organisms living in different environmental niches, for example at different depths in the soil profile (Kim et al., 2011); and (2) variation in (post-depositional) degradation of terrestrial end member biomarkers due to the differences in compound reactivities (e.g. Zhu et al., 2013). As BHT and adenosylhopane have different reactivity and therefore may degrade at different rates upon deposition (e.g. Cooke et al., 2008; Handley et al., 2010), this suggests, at least in some settings, that the R_{soil} could instead be used to describe relative rates of degradation.

4.3. *Biomarkers for aerobic methane oxidation*

Aminopentol (**1f**) is a biomarker for type I methanotrophs (Neunlist and Rohmer, 1985; Rohmer et al., 1984; Cvejic et al., 2000; Talbot et al., 2001; Coolen et al., 2008; van Winden et al., 2012a) with only one report of a non-methanotroph source, a species of *Desulfovibrio* sulfate reducing bacterium which had an extremely low concentration of aminopentol when grown in pure culture (Blumenberg et al., 2012). Concentrations of CH₄ oxidation markers (see Section 2.6 for definition; **1e**, **1f**, **4/5f**, **1f'**) varied throughout the samples here. High concentrations and relative abundances were present in the wetland samples, where aminopentol was the second most dominant BHP after BHT cyclitol ether (**1h**), confirming the occurrence of AMO (Table 6). The presence of CH₄ oxidation marker signatures suggests wetland environments as likely sources of these biomarkers in Congo fan sediments (Talbot et al., 2014) and therefore as sites of intense AMO within both modern and past climate phases. The data also agree with recent investigations of BHP signatures within the Amazon where Wagner et al. (2014) suggest wetland type environments as source areas for BHP CH₄ oxidation marker signatures. Thus, our Congo study is the second to document such a high abundance of CH₄ oxidation markers within tropical wetland samples (Fig. 4), suggesting that this might be a more general feature of tropical, and possibly other wetlands. This contrasts with the soil samples where aminotetrol was the most dominant CH₄ oxidation marker, but only a minor compound in the BHP suite overall (Table 3, 4 and 6). This was unexpected as 2 soils were sampled within an area of methane producing land cover (Fig. 2; swamp forest 11-1; tropical mixed forest 12-1), suggesting AMO should be a significant and readily identifiable from the BHP biomarker suite. Low levels of aminopentol and/or aminotetrol in soil samples could be due to low AMO activity in such samples. Alternatively, soil samples could have been collected when the oxic-anoxic boundary was shallowest. A study by van Winden et al. (2012a) found CH₄ oxidation markers in peatlands, specifically at the oxic-anoxic boundary where AMO is thought to occur. Additionally, Henckel et al. (2001) found that AMO increases during the drying out of

methane-producing wetland type environments, presumably due to the extension of the oxic-anoxic boundary. Lastly, the apparent lack of CH₄ oxidation markers in the soil samples could be due to a lack in our understanding of the source organisms of aminopentol and related compounds. Although many Type I methanotrophs make aminopentol as a dominant membrane component, followed by minor amounts of aminotetrol and aminotriol, other Type II methanotroph and at least one Type I methanotroph, *Methylobacterium album*, membranes are dominated by aminotetrol and aminotriol (e.g. Talbot et al., 2001; van Winden et al., 2012b and references therein).

4.4. BHP reservoirs

The Congo River drains the second largest basin in the world ($\sim 3.7 \times 10^6$ Km²). Soil derived OM is an important component of sediments deposited on the Congo fan (Holtvoeth et al., 2005). The organic fraction of ODP 1075 sediments relates to strongly degraded SOM of old highly developed, Kaolinite-rich feralitic soils (Oxisols) that cover large areas of the Congo river basin (Holtvoeth et al., 2005). The OC from the soils analysed in this study is transported through the Congo River and deposited in Malebo pool (Hughes et al., 2011; Spencer et al., 2012). Previous work has shown that OM exported from Malebo Pool is geochemically similar to OM at the head of the estuary (ca. 350 km downstream) and no major tributaries join the Congo River between this site and the Atlantic Ocean (Spencer et al., 2012). Similarity between the spread in R_{soil} indices for the soils and Malebo pool (Fig. 3) further suggests that BHPs are also subject to this transport mechanism. Due to the position of Malebo pool in the Congo River, OM and therefore BHPs signatures in the wetlands are representative of BHPs from the Congo watershed (Hughes et al., 2011; Spencer et al., 2012). Therefore, a terrestrial R_{soil} endmember of 0.63 (Malebo pool mean; Table 6) is representative of fluvially transported soils within the Congo watershed in combination with BHPs produced in Malebo Pool. Sediments deposited at Malebo pool are flushed into the estuary and then on to the Congo shelf and fan. As only one grab sample from the estuary was analysed in this study, the reported R_{soil} value of 0.2 (Table 6) may not represent the

true mean of the Congo estuary. However, BHT and adenosylhopane concentrations for ODP 1075 have previously been reported by Handley et al. (2010). Calculation of the R_{soil} index for sediments between 10 and 100 Ka ($n = 27$; Appendix II) show the R_{soil} index for the estuary is within the range of 0.16-0.54 (interglacial 0.16-0.54; glacial 0.21-0.52) for ODP 1075 sediments (Fig. 3). The mean R_{soil} index for ODP 1075 is 0.37 which is approximately half of the terrestrial end-member of Malebo pool, suggesting, that soil OM is a significant contributor to marine OM. This is in accordance with other studies from the Congo deep-sea fan. Holtvoeth et al. (2003) used a binary mixing model approach to determine that between 18 and 61% of bulk OM in ODP 1075 is of continental origin. Similarly, Weijers et al. (2009) used a 3 end-member mixing model to determine that between 38 and 52 % of OC within GeoB 6518-1 is of terrestrial (soil) origin.

Furthermore, strong similarities are found between the distribution of BHPs identified in the soils, wetlands, estuarine and ODP 1075 samples (Fig. 5 a,b). A suite of common BHPs are identified in the forest and savannah/field soils, and the wetlands, with more than half of the BHPs identified in the hinterland soils also identified in the wetlands. In addition, the common BHPs identified in the hinterland soils and the wetlands represent a major component of the soil BHP profile, contributing an average of 88% (forests) and 94% (savannah/field) of total BHPs (based on concentration; Fig. 5a). Lower BHP diversity is reported for samples from the Congo fan (Handley et al., 2010; Talbot et al., 2014) with many of the methylated and pentose compounds below detection limit. Between 7 and 10 of the 12 BHP compounds identified in ODP 1075 are also found in the wetlands and soils, and are again a major component of all of the BHP profiles representing over 90% of the total BHPs found in the wetland and estuary samples (Fig. 5b). Strong similarities between BHPs identified in the Congo hinterland and wetland samples and those identified in ODP 1075 suggests a link between BHP reservoirs. High concentrations of aminotetrol and aminopentol (including aminopentol isomer and unsaturated aminopentol) found in ODP 1075 sediments have previously been linked to fluvial transport of these biomarkers to the

Congo fan from Malebo pool and potentially similar wetlands (Talbot et al., 2014) with similar mechanisms also reported in the Amazon (Wagner et al., 2014). Due to the ubiquitous nature of BHT, aminotriol, BHT-, BHpentol and BHhexol cyclitol ether it is likely that the source of these compounds in the Congo fan will be both marine and terrestrial derived. The notable absence of methylated and unsaturated BHPs from ODP 1075 which represent no more than 18% of total BHPs in the soils and wetland sediments, is likely due to a dilution effect.

4.5. Trends in global BHP distribution

The data presented here suggest that BHP relative abundance may be controlled by large scale climate trends. Within the soils and wetlands from the Congo Basin, a narrow range in soil marker BHP relative abundance (7.9-36% of total BHPs) and tetrafunctionalised BHP relative abundance (52-81% of total BHPs) was observed (Fig. 4). The range is much smaller in comparison with studies from other less stable climatic zones, where surface soil marker BHP relative abundance varies between 0% and 66% of total BHPs and tetrafunctionalised BHP relative abundance varies between 34% and 100% of total BHPs (Cooke et al., 2008; Xu et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011; Fig. 4). Additionally, the mean soil marker BHP relative abundance for Congo soils (17%) is lower than that for temperate soils from northern and eastern Europe (28%; Cooke et al., 2008; Redshaw et al., 2008). High relative abundance of soil marker BHPs are found in soils from polar climates, with values between 27% and 55% of total BHPs for Svalbard (Rethemeyer et al., 2010) and 69-82% for surface and subsurface Yedoma permafrost from Siberia (Doğrul Selver et al., 2015). Xu et al. (2009) also observed abundances ranging from 35-52% of total BHPs in Alberta (Canada).

The differences may suggest that the main factors controlling BHP distributions in tropical climate zones are different from those from temperate and polar climate zones. Kim et al. (2011) found mean annual air temperature (MAAT) and precipitation to influence soil marker

BHP distribution in samples from the Mediterranean Têt watershed. Soils from the watershed were collected along a transect with strong environmental contrasts in altitude, MAAT, precipitation and a wide pH range, including some low pH peat samples. Kim et al. (2011) found that the lowest relative abundance of adenosylhopane (the dominant soil marker BHP) occurred at low altitude where MAAT was high, pH more alkaline and precipitation lowest. This could suggest that, during BHP synthesis, adenosylhopane (an intermediate in hopanoid biosynthesis; Bradley et al., 2010) is converted to other BHPs when environmental conditions are favourable for microbial activity (e.g. warmer).

The relationship between the structural diversity of BHPs and the role of these compounds within bacterial cells has not been fully elucidated. However, Poger and Mark (2013) suggest that BHPs may have a broader range in functionality within cell membranes than sterols within eukaryotes. Additionally, BHPs may be involved in a response to environmental stress (e.g. Kulkarni et al., 2013). The difference in BHP distributions between climate zones (Fig. 4) could suggest that, in addition to pH, environmental parameters such as seasonal temperature and precipitation may be important factors influencing BHP synthesis.

5. Conclusions

Up to 35 different BHPs were identified within 22 soils, 6 wetland and one estuarine sediment sample from the Congo. Dominant compounds in the soil and wetland samples were typically BHT, aminotriol and BHT cyclitol ether. However, BHP signatures produced by aerobic methane oxidising bacteria (including aminopentol and aminotetrol) were important within Malebo pool sediments and represented up to 26% of total BHPs. This indicates that taxonomic controls, in particular determining type and activity of aerobic methanotrophs, can be an important source of variability within the Congo samples.

Soil marker BHP relative abundances in the soils and wetland sediments were very similar. However, their relative proportion in the Congo soils (mean, 16% of total BHPs in forest soils;

19% of total BHPs in savannah/field soils) was lower than values for temperate and Arctic surface soils calculated from the available literature data. R_{soil} and R'_{soil} indices for the soils show a large range of 0.58-0.92 and 0.48-0.91, respectively, with savannah/field samples typically showing greater variation than forest soils. This is in accord with other R_{soil} and R'_{soil} values calculated from the literature and reinforces the need for local end members to be determined before any interpretation of the index values is undertaken.

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688

689 Figure Legends

690 **Fig. 1.** Structures of BHPs in Congo samples.

691 **Fig. 2.** Geographical locations of the study site in the Congo, showing the locations of 22 soil
692 samples (circles), 6 floodplain wetland sediment samples (Malebo pool; triangle), the Congo
693 estuary sediment sample (square) and ODP 1075 (star). The map is modified from Talbot et
694 al. (2014) and was generated using the planiglobe beta online plotting service
695 (<http://www.planiglobe.com>).

696 **Fig. 3.** Box plots showing range of R_{soil} values for soils and sediments including: forest soil
697 (this study; $n=16$); savanna/Field soil (this study; $n=6$); estuary (this study; $n = 1$); Congo fan
698 (ODP 1075) paleo sediments (Handley et al., 2010; $n=27$); wetland surface and subsurface
699 sediment (this study; $n=6$); Amazon wetlands (surface and subsurface; Wagner et al., 2014;
700 $n=5$); Amazon soil (Wagner et al., 2014; $n = 2$) San Salvador soils (Pearson et al., 2009;
701 $n=1$); Têt watershed surface soils (Kim et al., 2011; $n=12$); East China soil (Zhu et al., 2011;
702 $n=3$) Canadian surface soils (Xu et al., 2009; $n=5$); surface Permafrost (Rethemeyer et al.,
703 2010; $n=6$); Surface soils from Northern UK (Cooke et al., 2008; $n=4$). Further sample
704 information can be found in the supplementary data I.

705 **Fig. 4.** Ternary plot with relative abundance of tetrafunctionalised BHPs (%), sum of penta-
706 and hexafunctionalised BHPs (%) and soil marker BHPs (%) in Congo soils (this study; $n =$
707 22), Congo wetlands (this study; $n = 6$), Congo estuary sediment (this study; $n = 1$), Amazon
708 wetlands (surface and subsurface; Wagner et al., 2014; $n = 5$); Amazon soil (Wagner et al.,
709 2014; $n = 2$) San Salvador soils (Pearson et al., 2009; $n = 1$); Têt watershed surface soils
710 (Kim et al., 2011; $n = 12$); East China soil (Zhu et al., 2011; $n = 3$); Canadian surface soils
711 (Xu et al., 2009; $n = 5$); surface Permafrost (Rethemeyer et al., 2010; $n = 6$); Surface soils
712 from Northern UK (Cooke et al., 2008; $n = 4$). Further sample information can be found in the
713 supplementary data I.

Fig. 5. a; Mean number of BHPs identified in forest and savannah/field samples in common with wetlands (presented as % of total number of BHPs present; error bar represents 1 standard deviation) (white bars). Relative abundance of BHPs in forest and savannah/field samples in common with wetlands (black bars). b; Mean number of BHPs identified in forest, savannah/field, estuary and wetland samples in common with ODP 1075 (presented as % of total number of BHPs present; error bar represents 1 standard deviation) (white bars). Relative abundance of BHPs in forest, savannah/field, estuary and wetland samples in common with ODP 1075 (black bars).

725 **Table 1** Compounds in the samples and abbreviated names, structures and base peak (m/z) values.

Compound	Abbreviated name	Structure	Base peak m/z	Assignment
Anhydrobactriohopanetetrol	AnhdroBHT	1m	613	$[M+H]^+$
Ribonylhopane	Ribonylhopane	1k	627	$[M+H]^+$
Bacteriophane-32,33,34,35-tetrol	BHT	1b	655	$[M+H-CH_3COOH]^+$
2-methylbacteriophane-32,33,34,35-tetrol	2-methylBHT	2b	669	$[M+H-CH_3COOH]^+$
Bacteriophane-30,31,32,33,34,35-hexol	Bhhexol	1n	771	$[M+H-CH_3COOH]^+$
aminobacteriophene-32,33,34-triol	unsaturated aminotriol	4/5g	712	$[M+H]^+$
aminobacteriophane-32,33,34-triol	aminotriol	1g	714	$[M+H]^+$
2-methylaminobacteriophane-32,33,34-triol	2-methylaminotriol	2g	728	$[M+H]^+$
3-methylaminobacteriophane-32,33,34-triol	3-methylaminotriol	3g	728	$[M+H]^+$
35-aminobacteriophene-31,32,33,34-tetrol	unsaturated aminotetrol	4/5e	770	$[M+H]^+$
35-aminobacteriophane-31,32,33,34-tetrol	aminotetrol	1e	772	$[M+H]^+$
35-aminobacteriophene-30,31,32,33,34-pentol	unsaturated aminopentol	4/5f	828	$[M+H]^+$
35-aminobacteriophane-30,31,32,33,34-pentol	aminopentol	1f	830	$[M+H]^+$
35-aminobacteriophane-30,31,32,33,34-pentol isomer	aminopentol isomer	1f'	788	$[M+H]^+$
30-(5'-adenosyl)hopane	G1	1a	788	$[M+H]^+$
2-methyl-30-(5'-adenosyl)hopane	2-Me G1	2a	802	$[M+H]^+$
Adenosylhopane type 2	G2	1c	761	$[M+H]^+$
2-methyladenosylhopane type 2	2-Me G2	2c	775	$[M+H]^+$
Adenosylhopane type 3	G3	1d	802	$[M+H]^+$
2-Methyladenosylhopane type 3	2-Me G3	2d	816	$[M+H]^+$
Bacteriophene-32,33,34,35-tetrol pseudopentose	unsaturated BHTpentose	4/5l	941	$[M+H-CH_3COOH]^+$
Bacteriophane-32,33,34,35-tetrol pseudopentose	BHTpentose	1l	943	$[M+H-CH_3COOH]^+$
2-methylbacteriophane-32,33,34,35-tetrol pseudopentose	2-methylBHTpentose	2l	957	$[M+H-CH_3COOH]^+$
Bacteriophanetetrol carbopseudopentose ether	BHT cyclitol ether	1h	1002	$[M+H]^+$
2-methylbacteriophanetetrol carbopseudopentose ether	BHT cyclitol ether isomer	1h	1002	$[M+H]^+$
Bacteriophanetetrol carbopseudopentose ether	2-methylBHT cyclitol ether	2h	1016	$[M+H]^+$
Bacteriophanetetrol carbopseudopentose ether	3-methylBHT cyclitol ether	3h	1016	$[M+H]^+$
Bacteriophanetetrol carbopseudopentose ether glucosamine	BHT glucosamine	1o	1002	$[M+H]^+$
Bacteriophanepentol carbopseudopentose ether	BHpentol cyclitol ether	1i	1060	$[M+H]^+$
Bacteriophanepentol carbopseudopentose ether (isomer)	BHpentol cyclitol ether (isomer)	1i	1060	$[M+H]^+$
2-methylbacteriophanepentol carbopseudopentose ether (isomer)	2-methylBHpentol cyclitol ether	2i	1074	$[M+H]^+$
3-methylbacteriophanepentol carbopseudopentose ether (isomer)	3-methylBHpentol cyclitol ether	3i	1074	$[M+H]^+$
Bacteriophane-30,31,32,33,34,35-hexol carbopseudopentose ether	Bhhexol cyclitol ether	1j	1118	$[M+H]^+$
Bacteriophane-30,31,32,33,34,35-hexol carbopseudopentose ether (isomer)	Bhhexol cyclitol ether (isomer)	1j	1118	$[M+H]^+$
2-methylbacteriophane-30,31,32,33,34,35-hexol carbopseudopentose ether	2-methylBhhexol cyclitol ether	2j	1132	$[M+H]^+$
3-methylbacteriophane-30,31,32,33,34,35-hexol carbopseudopentose ether	3-methylBhhexol cyclitol ether	3j	1132	$[M+H]^+$

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728 **Table 2**729 Soil and sediment sample names, corresponding abbreviated names with TOC (%), pH, R_{soil} 730 and R'_{soil} (nm, not measured).

	Sample	Abbreviated name	TOC (%)	pH	R_{soil}	R'_{soil}
Forest	Closed evergreen lowland forest	CELF JP6	3.4	3.82	0.92	0.91
	Closed evergreen lowland forest	CELF C6B	0.23	4.71	0.72	0.68
	Closed evergreen lowland forest	CELF C17B	1.08	4.6	0.85	0.84
	Closed evergreen lowland forest	CELF C18B	1.59	4.31	0.83	0.81
	Closed evergreen lowland forest	CELF C19B	2.04	3.73	0.89	0.86
	Closed evergreen lowland forest	CELF C27B	4.48	3.97	0.85	0.81
	Logged tropical mixed forest	LTF 7-1	2.95	3.78	0.74	0.73
	Logged tropical mixed forest	LTF 8-1	2.6	3.66	0.83	0.82
	Logged tropical mixed forest	LTF 10-1	1.47	3.09	0.8	0.78
	Tropical mixed forest	TMF 12-1	2.68	3.61	0.73	0.71
	Gilbertiodendron forest	GF 9-1	6.11	3.81	0.7	0.69
	Swamp forest	SF 11-1	2.51	3.72	0.8	0.78
	Tropical seasonally flooded forest	TSFF 6-1	1.28	4.57	0.63	0.61
	Secondary forest in savanna-forest mosaic	SFS 3-1	2.23	3.76	0.88	0.86
	Field in savanna-forest mosaic	FSFM 4-1	1.23	4.72	0.64	0.6
	Mosaic Forest/Croplands	MF C8B	0.71	4.74	0.84	0.82
	Swamp bushland and grassland	SB C38B	1.26	5.21	0.87	0.83
Savannah/Field	Closed grassland	CG C46B	2.05	5.07	0.8	0.73
	Savanna outside of BZV	SBZV 1-1	0.36	5.75	0.65	0.6
	Scrub savanna	SS 1-1	0.6	4.58	0.6	0.54
	Scrub savanna	SS 5-1	1.17	3.98	0.58	0.48
	Field	F 13-1	1.07	4.36	0.78	0.74
Wetland	Permanently submerged sediment	PS 0-5	1.34	4.27	0.63	0.59
	Permanently submerged sediment	PS 5-15	1.32	4.53	0.65	0.62
	Recently exposed sediment	RE 0-5	2.68	nm	0.63	0.59
	Recently exposed sediment	RE 5-15	2.51	4.38	0.66	0.62
	Exposed floodplain with occasional submersion	EF 0-5	1.1	4.47	0.63	0.6
	Exposed floodplain with occasional submersion	EF 5-15	1.62	4.8	0.61	0.59
	Estuary	Estuary	2.9	nm	0.2	0.2

731

732 **Table 3**733 Concentration ($\mu\text{g/g}$ TOC) of bacteriohopanepolyols in 16 forest soils from the Congo (bdl,

734 below detection limit).

Structure	CELF JP6	CELF C6B	CELF C17B	CELF C18B	CELF C19B	CELF C27B	LTF 7-1	LTF 8-1	LTF 10-1	TMF 12-1	GF 9-1	SF 11-1	TSFF 6-1	SFS 3-1	FSFM 4-1	MF C8B
1m	bdl	bdl	bdl	bdl	bdl	bdl	10	5.0	6.1	8.7	5.3	10	20	bdl	bdl	bdl
1k	bdl	bdl	8.0	6.1	6.2	2.0	11	19	15	8.6	20	16	12	5.6	5.4	bdl
1b	22	34	55	64	71	30	230	190	210	130	270	210	390	79	180	55
2b	13	4.1	18	24	42	11	81	130	130	45	160	63	52	46	26	15
1n	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4/5g	96	28	92	55	270	69	70	66	110	35	21	240	110	16	22	240
1g	940	270	740	720	1300	440	790	1600	1500	590	1300	770	730	340	540	1000
2g	22	8.3	20	30	42	19	16	40	25	10	31	19	7.6	9	12	26
3g	5.3	2.3	5.6	8.7	5.4	6.0	bdl	30	28	14	21	19	21	bdl	7.7	9.4
4/5e	bdl	bdl	bdl	bdl	7.7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1e	42	18	38	81	87	16	26	59	67	17	23	47	30	8.6	16	24
4/5f	1.2	bdl	bdl	13	1.9	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1f	bdl	13	47	260	14	13	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	11
1f'	bdl	bdl	bdl	55	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1a	200	59	180	170	300	69	570	800	700	290	540	580	520	380	230	120
2a	19	8.0	13	15	37	2.9	35	69	52	17	34	53	37	52	25	13
1c	20	8.7	95	100	130	56	35	28	44	22	48	130	55	91	20	130
2c	4.8	4.8	17	24	60	37	5.9	8	10	5.8	12	48	15	19	15	10
1d	5.5	5.1	10	6.1	16	bdl	17	27	19	12	bdl	22	24	16	22	8.9
2d	bdl	1.1	1.9	3.5	4.4	bdl	7.3	26	14	4.1	bdl	bdl	12	6.9	14	5.6
4/5l	bdl	bdl	bdl	bdl	bdl	bdl	33	57	68	19	35	27	58	15	38	bdl
1l	24	10	26	28	20	15	86	100	120	44	130	30	110	16	30	27
2l	bdl	bdl	bdl	bdl	bdl	bdl	30	100	26	26	96	bdl	bdl	bdl	35	bdl
1h	260	250	270	970	740	400	870	1400	1500	530	1000	1400	1600	360	290	390
1h	120	bdl	360	bdl	370	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	270
2h	120	23	89	100	220	97	84	290	360	97	86	230	170	bdl	bdl	120
3h	bdl	4.6	bdl	11	42	6.1	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	22
1o	bdl	9.2	25	24	23	7.5	23	25	48	13	24	bdl	25	11	bdl	14
1i	76	54	120	95	190	53	170	180	270	95	210	180	340	43	55	110
1i	20	12	77	18	44	14	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	38
2i	10	3.2	12	5.4	12	5.9	40	71	73	24	35	32	56	19	bdl	5.9
3i	bdl	bdl	bdl	bdl	bdl	bdl	23	57	40	6	18	22	10	21	41	bdl
1j	92	26	41	54	100	37	150	280	280	100	190	240	340	25	42	55
1j	bdl	6.6	12	5.4	22	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	7.1
2j	bdl	bdl	bdl	4.5	3.3	bdl	14	57	bdl	12	bdl	bdl	bdl	bdl	bdl	3.1
3j	19	bdl	3.0	4.2	8.8	1.7	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	2.8

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736

737 **Table 4**

738 Concentration (µg/g TOC) of bacteriohopanepolyols in 6 Savannah/field soils from the

739 Congo (bdl, below detection limit).

Structure	SB C38B	CG C46B	SBZV 1-1	SS 1-1	SS 5-1	F 13-1
1m	bdl	bdl	bdl	bdl	bdl	bdl
1k	bdl	bdl	bdl	bdl	bdl	bdl
1b	14	30	160	160	76	220
2b	4.2	5.0	24	32	24	74
1n	bdl	bdl	bdl	bdl	bdl	bdl
4/5g	11	38	9.1	20	42	31
1g	300	550	210	410	480	360
2g	17	15	10	bdl	20	11
3g	2.0	7.3	bdl	bdl	bdl	bdl
4/5e	1.8	bdl	bdl	bdl	bdl	bdl
1e	19	14	bdl	2.1	12	15
4/5f	bdl	bdl	bdl	bdl	bdl	bdl
1f	0.92	bdl	bdl	bdl	bdl	bdl
1f'	bdl	bdl	bdl	bdl	bdl	bdl
1a	47	61	200	160	33	460
2a	10	10	38	36	21	84
1c	23	10	26	18	25	140
2c	13	26	26	18	16	71
1d	bdl	8.2	13	13	11	16
2d	bdl	3.7	bdl	bdl	bdl	bdl
4/5l	bdl	bdl	bdl	bdl	22	49
1l	bdl	3.9	bdl	bdl	bdl	bdl
2l	bdl	bdl	bdl	bdl	bdl	bdl
1h	50	200	97	250	370	900
1h	47	bdl	bdl	bdl	bdl	bdl
2h	3.3	29	bdl	45	bdl	130
3h	2.6	9.2	bdl	bdl	bdl	bdl
1o	bdl	2.1	bdl	bdl	bdl	19
1i	39	42	12	23	55	140
1i	7.0	11	bdl	bdl	bdl	bdl
2i	1.6	4.3	6.7	14	bdl	42
3i	bdl	bdl	11	48	67	31
1j	10	18	bdl	bdl	70	120
1j	bdl	2.2	bdl	bdl	bdl	bdl
2j	bdl	0.48	bdl	bdl	bdl	13
3j	0.9	1.2	bdl	bdl	bdl	bdl

740 **Table 5**

741 Concentration (µg/g TOC) of bacteriohopanepolyols in 6 Malebo pool wetland and 1
742 estuarine sediment from the Congo (bdl, below detection limit).

Structure	PS 0-5	PS 5-10	RE 0-5	RE 5-10	EF 0-5	EF 5-10	Estuary
1m	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1k	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1b	490	590	400	370	460	390	320
2b	53	84	70	81	41	36	24
1n	40	65	46	49	33	34	bdl
4/5g	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1g	960	1100	420	360	950	710	320
2g	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3g	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4/5e	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1e	270	270	110	89	230	200	82
4/5f	58	56	31	27	58	52	12
1f	1200	1100	640	500	1200	1100	180
1f'	69	86	64	51	70	86	68
1a	640	910	520	560	640	520	81
2a	100	110	100	91	64	50	bdl
1c	45	48	31	33	37	29	bdl
2c	17	19	22	13	11	5.6	bdl
1d	16	23	14	13	18	14	bdl
2d	bdl	bdl	bdl	bdl	bdl	bdl	bdl
4/5l	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1l	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2l	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1h	2000	2200	1200	1300	1700	1700	230
1h	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2h	230	260	170	170	130	120	bdl
3h	bdl	bdl	bdl	bdl	bdl	bdl	bdl
1o	bdl	bdl	bdl	bdl	bdl	bdl	12
1i	280	250	190	220	170	220	33
1i	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2i	38	41	42	29	12	15	bdl
3i	59	59	38	48	18	23	bdl
1j	260	250	220	280	200	260	21
1j	bdl	bdl	bdl	bdl	bdl	bdl	bdl
2j	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3j	bdl	bdl	bdl	bdl	bdl	bdl	bdl

Table 6

Summary of CH₄ oxidation markers and soil marker BHPs (% , relative total BHPs), R_{soil} and R'_{soil} plus literature data on surface soil and peat BHP composition.

Location	N	CH ₄ oxidation markers (%)		Soil marker BHPs (%)		R_{soil} (R'_{soil})		Reference
		Mean	Range	Mean	Range	Mean	Range	
Congo Forest soils	16	2.3	0.53 - 14	16	10 - 36	0.79 (0.77)	0.63 - 0.92 (0.60 - 0.91)	This study
Congo savannah/fields	6	1.0	0 - 3.2	19	7.9 - 36	0.71 (0.65)	0.58 - 0.87 (0.48 - 0.83)	This study
Congo wetlands (surface and subsurface)	6	22	16 - 26	14	11 - 17	0.63 (0.60)	0.61 - 0.66 (0.59 - 0.62)	This study
Amazon soils	2	4.3	0.94 - 7.7	23	18 - 28	0.64 (0.61)	0.44 - 0.84 (0.41 - 0.81)	Wagner et al., 2014
Amazon wetlands (surface and subsurface)	5	37	24 - 45	6.0	2.6 - 11	0.45 (0.43)	0.27 - 0.68 (0.21 - 0.64)	Wagner et al., 2014
Tropical soil San Salvador	1	5.8		21		0.48 (0.48)		Pearson et al., 2009
Têt (surface soils)	12	1.0	0 - 5.8	41	0 - 66	0.54 (0.52)	0 - 0.87 (0 - 0.85)	Kim et al., 2010
Têt peat (surface)	2	1.3	0 - 2.5	27	24 - 31	0.62 (0.60)	0.53 - 0.71 (0.51 - 0.68)	Kim et al., 2010
East China (Mid catchment surface soils)	3	2.4	0.52 - 6.1	20	12 - 30	0.74 (0.70)	0.60 - 0.82 (0.57 - 0.80)	Zhu et al., 2011
Canada	5	1.4	0.96 - 2.0	43	35 - 52	0.79 (0.76)	0.67 - 0.85 (0.65 - 0.81)	Xu et al., 2009
Arctic permafrost	6	0	0	40	27 - 55	0.64 (0.60)	0.53 - 0.75 (0.48 - 0.72)	Rethemeyer et al., 2010
Northern UK (surface)	4	0.85	0 - 2.0	23	20 - 27	0.48 (0.42)	0.36 - 0.64 (0.30 - 0.58)	Cooke et al., 2008

Supplementary information

–Data citation

We integrated published BHP data to make Fig. 3 and 4 and Table 6. Amazon Basin soils are cited from Wagner et al., (2014) and include sample 5 (seasonally inundated soil, 0-20 cm depth), sample 6 (Terra Firme soil, 0-20 cm). Amazon wetland samples are cited from Wagner et al., (2014) and include sample 1 (wetland sediment, 0-10 and 200-250 cm), sample 2 (wetland sediment, 0-10 and 25-30 cm), sample 3 (wetland sediment, 0-25 cm). San Salvador upland soil is cited from Pearson et al. (2009). Têt watershed soil is cited from Kim et al. (2010) and includes TESO1 (2-7 cm), TESO5 (2-7 cm), TESO15 (1-5 cm), TESO17 (upper 10 cm), TESO19 (upper 10 cm), TESO 32 (upper 10 cm), TESO35 (upper 10 cm), TESO36 (0-5 cm), TESO39 (upper 10 cm), TESO41 (upper 10 cm), TESO47 (10 cm), TESO 48 (0-20 cm). Têt watershed peat samples are cited from Kim et al. (2010) and include TESO2 (0-10 cm), TESO49 (0-10 cm). Soils from east China are cited from Zhu et al. (2011) and include arable farm, grassland, afforestation. Canadian soils are cited from Xu et al. (2009) and include grassland soil 1 Ah horizon (0-15 cm), grassland soil 2 Ah horizon (0-15cm), grassland 3 Ah horizon (0-15 cm), aspen forest-grassland transition soil Ah horizon (0-10 cm), aspen forest soil O horizon (0-15 cm). Arctic permafrost soils are cited from Rethemeyer et al. (2010) and include Kolhaugen Hill trough (0-30 cm), Kolhaugen Hill centre (0-30 cm), Leierhaugen Hill (0-30 cm), upper drainage area (0-20cm), upper drainage area (0-20 cm), lower drainage area (0-20 cm). Northern UK soils are cited from Cooke et al. (2008) and include university garden DSNM (0-10 cm), arable TFWC (0-10 cm), pasture WNBC9 (0-10 cm), Palace Leas plot 7 (0-5 cm).