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## Bacteriohopanepolyols in tropical soils and sediments from 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 bacteriohopanepolyol (BHP) inventory of a suite of tropical soils and, from comparison with published data, propose some initial ideas on BHP distribution controls. Strong taxonomic controls on BHP production are evident in wetland sediments. Dominant within the suite were 35-aminobacteriohopane-31,32,33,34-tetrol (aminotetrol) and 35-aminobacteriohopane-30,31,32,33,34-pentol (aminopentol), indicating aerobic methanotrophy. A narrow range and low mean relative abundance of 30-(5'-adenosyl)hopane (adenosylhopane) and related compounds, collectively termed “soil marker” BHPs, were 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.

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### 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), 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; 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., 2007, 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), but it is unknown why accumulation of adenosylhopane occurs only 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 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)

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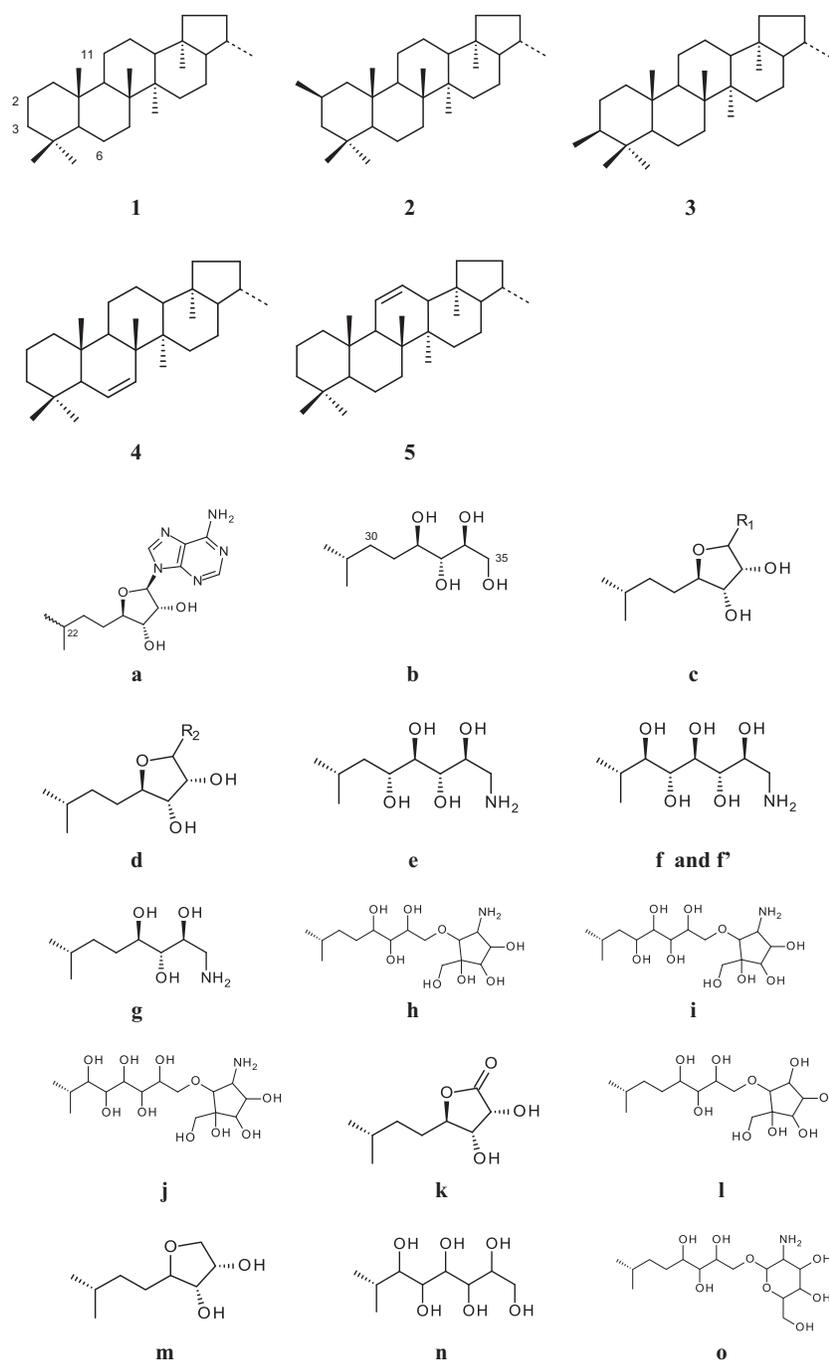


Fig. 1. Structures of BHPs in Congo samples.

transport (Cooke et al., 2008b, 2009; Zhu et al., 2011; Doğrusel Selver et al., 2012, 2015). Another group of diagnostic markers comprises 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) suggest aminopentol in sediments dating back 30 Ka from the Amazon fan, is derived from the Amazon

catchment, with fluctuation 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 have detailed 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 (Talbot and Farrimond, 2007; Coolen et al., 2008; Cooke et al., 2008b; Blumenberg et al., 2010; Zhu et al., 2011), with the exception of deep sea-fan sediments with very high terrigenous 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 in two of four surface soils from the northern UK), and Zhu et al. (2011) up to 20 BHPs 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 concentration and greatest structural diversity within wetland sediments (18 in sediments vs. 13 in the soils; Wagner et al., 2014), suggesting wetlands as possibly a significant source of BHPs in shelf and fan systems. As tropical wetlands and soils are largely understudied, significant 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). Studies of sediments from the Congo fan suggest 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.

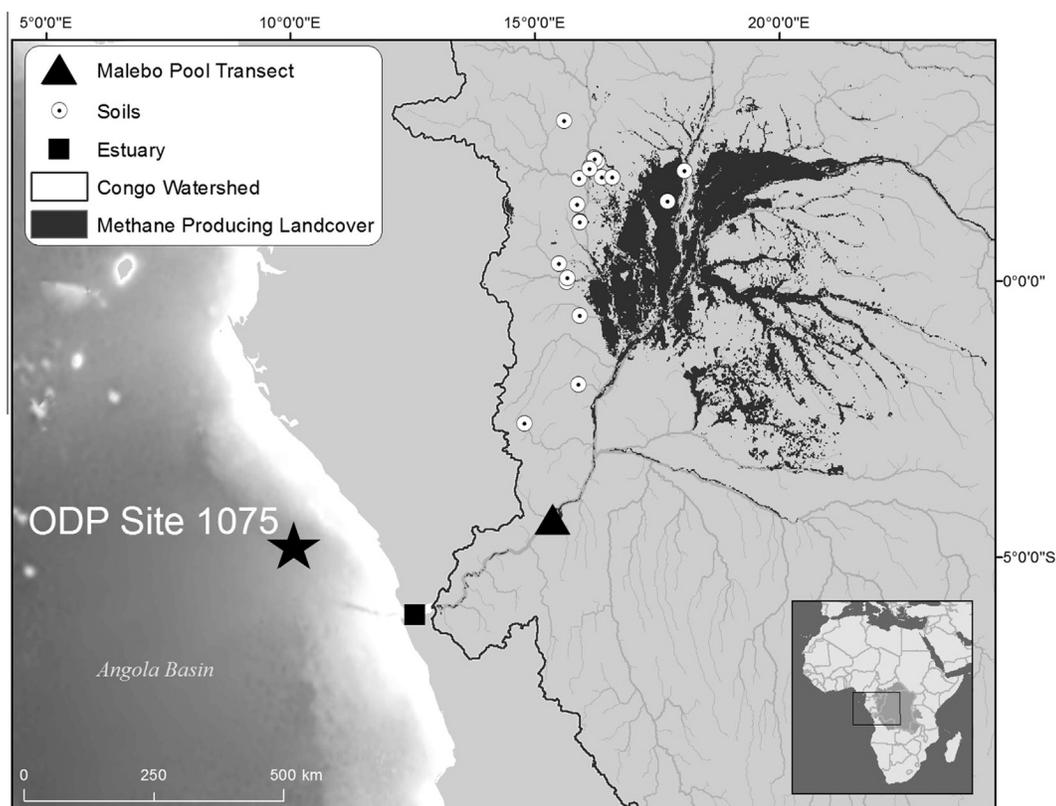
## 2. Material and methods

### 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, stored frozen on arrival and freeze dried and ground prior to extraction.

Malebo Pool flood plain 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 (UK).



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

## 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 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. Ca. 0.1 g sample were treated with 4 mol/l HCl (60–70 °C) for removal of inorganic carbon, after which, the HCl was allowed to drain. 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 h. TOC was measured (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  $\pm 0.05\%$  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/CHCl<sub>3</sub> (4:10:5, v/v). The mixture was sonicated (40 °C, 1 h) followed by shaking at room temperature (2–4 h). It was centrifuged at 12,000 rpm (15 min) and the supernatant transferred to a second centrifuge tube. This process was repeated 3 $\times$ . The decanted supernatant was phase separated using CHCl<sub>3</sub> (5 ml) and water (5 ml). The tubes containing the supernatants were centrifuged for 5 min to complete the separation of the organic (CHCl<sub>3</sub>) and MeOH/water phases. The combined organic (CHCl<sub>3</sub>) 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) CHCl<sub>3</sub>/MeOH (2:1, v/v). The total lipid extract (TLE) was evaporated to dryness under a stream of N<sub>2</sub>. A 5 $\alpha$ -pregnane-3 $\beta$ ,20 $\beta$ -diol internal standard was added (0.236  $\mu$ g/ $\mu$ l) and the TLE split into 3 equal aliquots following dilution with 5 ml CHCl<sub>3</sub>/MeOH (2:1, v/v; heated at 50 °C for 10 min).

## 2.5. BHP analysis

One third of the TLE was used: the aliquot was evaporated to dryness under N<sub>2</sub> and acetylated by adding Ac<sub>2</sub>O (1 ml) and pyridine (1 ml), heating (1 h, 50 °C) and leaving at room temperature overnight. The Ac<sub>2</sub>O and pyridine were removed under a stream of N<sub>2</sub> and the resulting acetylated extract was dissolved in 1 ml MeOH/propan-2-ol (3:2, v/v).

BHP analysis was performed using reversed phase high performance liquid chromatography-atmospheric pressure chemical ionisation-mass spectrometry (HPLC-APCI-MS<sup>n</sup>) with a ThermoFinnigan surveyor HPLC system fitted with a Phenomenex Gemini C<sub>18</sub> column (150 mm; 3.0 mm i.d.; 5  $\mu$ 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% H<sub>2</sub>O (0 min); 59% MeOH, 1% H<sub>2</sub>O, 40% propan-2-ol (at 25 min); isocratic to 45 min, returning to 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  $\mu$ A, and sheath and auxiliary gas flow of 40 and 10, respectively (arbitrary units). MS<sup>n</sup> analysis was carried out in data dependent mode with three scan events: SCAN 1: full spectrum, *m/z* 300–1300; SCAN 2: data dependent MS<sup>2</sup> spectrum of most intense ion from SCAN 1; SCAN 3: data-dependent MS<sup>3</sup> 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 by employing the characteristic base peak ion areas of individual BHPs in mass chromatograms (from SCAN 1) relative to the *m/z* 345 chromatogram base peak area of the acetylated 5 $\alpha$ -pregnane-3 $\beta$ ,20 $\beta$ -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 C-32, C-33, C-34 and C-35 ([Fig. 1](#)). Pentafunctionalised compounds have an additional functional group at C-31 and hexafunctionalised compounds have 2 additional functional groups at C-30 and C-31.

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

The *R*<sub>soil</sub> index (as defined by [Zhu et al., 2011](#)) was calculated according to the relative concentrations of BHT (**1b**) and all soil marker BHPs. The *R'*<sub>soil</sub> index was later proposed as an alternative index excluding methylated homologues for settings where the C-2 methylated soil marker BHPs were infrequently/intermittently present ([Doğrul Selver et al., 2012](#)) and is calculated according to the relative concentrations of BHT (**1b**) and adenosylhopane (**1a**), adenosylhopane type 2 (**1c**) and adenosylhopane type 3 (**1d**).

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

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

AminobHPs include aminotriol (**1g**), unsaturated (**4/5g**) and methylated aminotriol (**2/3g**), aminotetrol (**1e**) and unsaturated aminotetrol (**4/5e**), and aminopentol (**1f**), unsaturated (**4/5f**) and aminopentol isomer (**1f'**; [van Winden et al., 2012a](#)). BHPs diagnostic for aerobic methane oxidation (hereafter “CH<sub>4</sub> oxidation markers”) include aminotetrol (**1e**), aminopentol (**1f**), unsaturated (**4/5f**) and aminopentol isomer (**1f'**; [van Winden et al., 2012a](#)).

The data were found to have a non-parametric distribution and were not mathematically transformed prior to statistical analysis.

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

Compound	Abbreviated name	Structure	Base peak <i>m/z</i>	Assignment
Anhydrobacteriohopanetetrol	AnhdroBHT	<b>1m</b>	613	[M+H] <sup>+</sup>
Ribonylhopane	Ribonylhopane	<b>1k</b>	627	[M+H] <sup>+</sup>
Bacteriohopane-32,33,34,35-tetrol	BHT	<b>1b</b>	655	[M+H-CH <sub>3</sub> COOH] <sup>+</sup>
2-methylbacteriohopane-32,33,34,35-tetrol	2-methylBHT	<b>2b</b>	669	[M+H-CH <sub>3</sub> COOH] <sup>+</sup>
Bacteriohopane-30,31,32,33,34,35-hexol	Bhhexol	<b>1n</b>	771	[M+H-CH <sub>3</sub> COOH] <sup>+</sup>
Aminobacteriohopene-32,33,34-triol	Unsaturated aminotriol	<b>4/5g</b>	712	[M+H] <sup>+</sup>
Aminobacteriohopane-32,33,34-triol	Aminotriol	<b>1g</b>	714	[M+H] <sup>+</sup>
2-methylaminobacteriohopane-32,33,34-triol	2-methylaminotriol	<b>2g</b>	728	[M+H] <sup>+</sup>
3-methylaminobacteriohopane-32,33,34-triol	3-methylaminotriol	<b>3g</b>	728	[M+H] <sup>+</sup>
35-aminobacteriohopene-31,32,33,34-tetrol	Unsaturated aminotetrol	<b>4/5e</b>	770	[M+H] <sup>+</sup>
35-aminobacteriohopane-31,32,33,34-tetrol	Aminotetrol	<b>1e</b>	772	[M+H] <sup>+</sup>
35-aminobacteriohopene-30,31,32,33,34-pentol	Unsaturated aminopentol	<b>4/5f</b>	828	[M+H] <sup>+</sup>
35-aminobacteriohopane-30,31,32,33,34-pentol	Aminopentol	<b>1f</b>	830	[M+H] <sup>+</sup>
35-aminobacteriohopane-30,31,32,33,34-pentol isomer	Aminopentol isomer	<b>1f'</b>	788	[M+H] <sup>+</sup>
30-(5'-adenosyl)hopane	G1	<b>1a</b>	788	[M+H] <sup>+</sup>
2-methyl-30-(5'-adenosyl)hopane	2-Me G1	<b>2a</b>	802	[M+H] <sup>+</sup>
Adenosylhopane type 2	G2	<b>1c</b>	761	[M+H] <sup>+</sup>
2-methyladenosylhopane type 2	2-Me G2	<b>2c</b>	775	[M+H] <sup>+</sup>
Adenosylhopane type 3	G3	<b>1d</b>	802	[M+H] <sup>+</sup>
2-Methyladenosylhopane type 3	2-Me G3	<b>2d</b>	816	[M+H] <sup>+</sup>
Bacteriohopene-32,33,34,35-tetrol pseudopentose	Unsaturated BHTpentose	<b>4/5l</b>	941	[M+H-CH <sub>3</sub> COOH] <sup>+</sup>
Bacteriohopane-32,33,34,35-tetrol pseudopentose	BHTpentose	<b>1l</b>	943	[M+H-CH <sub>3</sub> COOH] <sup>+</sup>
2-methylbacteriohopane-32,33,34,35-tetrol pseudopentose	2-methylBHTpentose	<b>2l</b>	957	[M+H-CH <sub>3</sub> COOH] <sup>+</sup>
Bacteriohopanetetrol carbopseudopentose ether	BHT cyclitol ether	<b>1h</b>	1002	[M+H] <sup>+</sup>
2-methylbacteriohopanetetrol carbopseudopentose ether	BHT cyclitol ether isomer	<b>1h'</b>	1002	[M+H] <sup>+</sup>
Bacteriohopanetetrol carbopseudopentose ether	2-methylBHT cyclitol ether	<b>2h</b>	1016	[M+H] <sup>+</sup>
Bacteriohopanetetrol carbopseudopentose ether	3-methylBHT cyclitol ether	<b>3h</b>	1016	[M+H] <sup>+</sup>
Bacteriohopanetetrol carbopseudopentose ether glucosamine	BHT glucosamine	<b>1o</b>	1002	[M+H] <sup>+</sup>
Bacteriohopanepentol carbopseudopentose ether	BHpentol cyclitol ether	<b>1i</b>	1060	[M+H] <sup>+</sup>
Bacteriohopanepentol carbopseudopentose ether (isomer)	BHpentol cyclitol ether (isomer)	<b>1i'</b>	1060	[M+H] <sup>+</sup>
2-methylbacteriohopanepentol carbopseudopentose ether (isomer)	2-methylBHpentol cyclitol ether	<b>2i</b>	1074	[M+H] <sup>+</sup>
3-methylbacteriohopanepentol carbopseudopentose ether (isomer)	3-methylBHpentol cyclitol ether	<b>3i</b>	1074	[M+H] <sup>+</sup>
Bacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether	Bhhexol cyclitol ether	<b>1j</b>	1118	[M+H] <sup>+</sup>
Bacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether (isomer)	Bhhexol cyclitol ether (isomer)	<b>1j'</b>	1118	[M+H] <sup>+</sup>
2-methylbacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether	2-methylBHhexol cyclitol ether	<b>2j</b>	1132	[M+H] <sup>+</sup>
3-methylbacteriohopane-30,31,32,33,34,35-hexol carbopseudopentose ether	3-methylBHhexol cyclitol ether	<b>3j</b>	1132	[M+H] <sup>+</sup>

Spearman's rho ( $r_s$ ) was calculated using IBM SPSS 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 (Tables 3 and 4). Aminotriol (**1g**) and BHT cyclitol ether (**1h**) were 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 BHpentolcyclitol ethers (**2i** and **3i**) and BHhexolcyclitol (**2j** and **3j**) ethers were also present in the soil samples, though as minor components (Tables 3 and 4).

Aminopentol (**1f**) was a minor component of the BHP suite, with a concentration ranging from 0.92–47 µg/g TOC within six soils. However, it 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 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 (Tables 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. 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



**Table 4**Concentration ( $\mu\text{g/g}$  TOC) of bacterioplanepolyols in 6 Savannah/field soils from the Congo (bdl, below detection limit).

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

**Table 5**Concentration ( $\mu\text{g/g}$  TOC) of bacterioplanepolyols in 6 Malebo pool wetland and 1 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
<b>1m</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1k</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1b</b>	490	590	400	370	460	390	320
<b>2b</b>	53	84	70	81	41	36	24
<b>1n</b>	40	65	46	49	33	34	bdl
<b>4/5g</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1g</b>	960	1100	420	360	950	710	320
<b>2g</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>3g</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>4/5e</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1e</b>	270	270	110	89	230	200	82
<b>4/5f</b>	58	56	31	27	58	52	12
<b>1f</b>	1200	1100	640	500	1200	1100	180
<b>1f'</b>	69	86	64	51	70	86	68
<b>1a</b>	640	910	520	560	640	520	81
<b>2a</b>	100	110	100	91	64	50	bdl
<b>1c</b>	45	48	31	33	37	29	bdl
<b>2c</b>	17	19	22	13	11	5.6	bdl
<b>1d</b>	16	23	14	13	18	14	bdl
<b>2d</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>4/5l</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1l</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>2l</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1h</b>	2000	2200	1200	1300	1700	1700	230
<b>1h'</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>2h</b>	230	260	170	170	130	120	bdl
<b>3h</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>1o</b>	bdl	bdl	bdl	bdl	bdl	bdl	12
<b>1i</b>	280	250	190	220	170	220	33
<b>1i'</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>2i</b>	38	41	42	29	12	15	bdl
<b>3i</b>	59	59	38	48	18	23	bdl
<b>1j</b>	260	250	220	280	200	260	21
<b>1j'</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>2j</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl
<b>3j</b>	bdl	bdl	bdl	bdl	bdl	bdl	bdl

dominant along with BHT cyclitol ether (**1h**) and aminotriol (**1g**) (Table 3). The wetland sediments also contained other  $\text{CH}_4$  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  $\mu\text{g/g}$  TOC (exposed with occasional flooding sub surface sample; EF 5-15) to 1100  $\mu\text{g/g}$  TOC (PS 5-15). Relative abundance of soil marker BHPs ranged from 11–17% of total BHPs.  $R_{\text{soil}}$  index ranged from 0.61–0.66 (avg. 0.63; Fig. 3) and the  $R'_{\text{soil}}$  index from 0.59–0.62 (avg. 0.60) (Table 2).

### 3.4. Estuarine sediment

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

## 4. Discussion

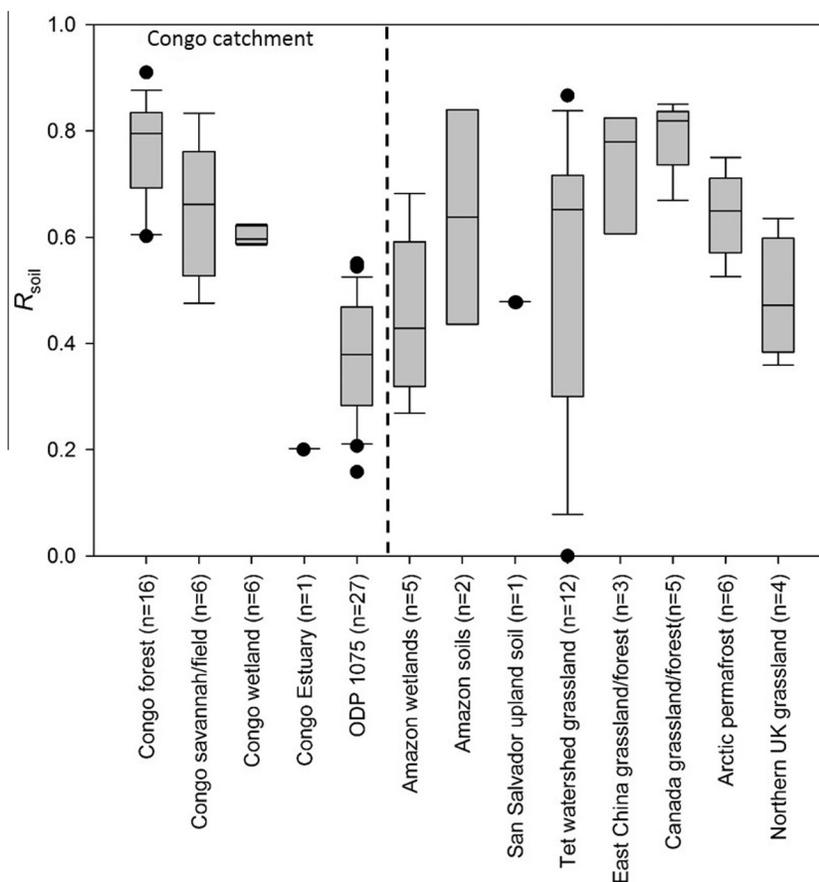
### 4.1. BHP distributions

The soils were dominated by non-source specific BHPs (Tables 3–5). Greater 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

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%; 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., 2008a; 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;



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

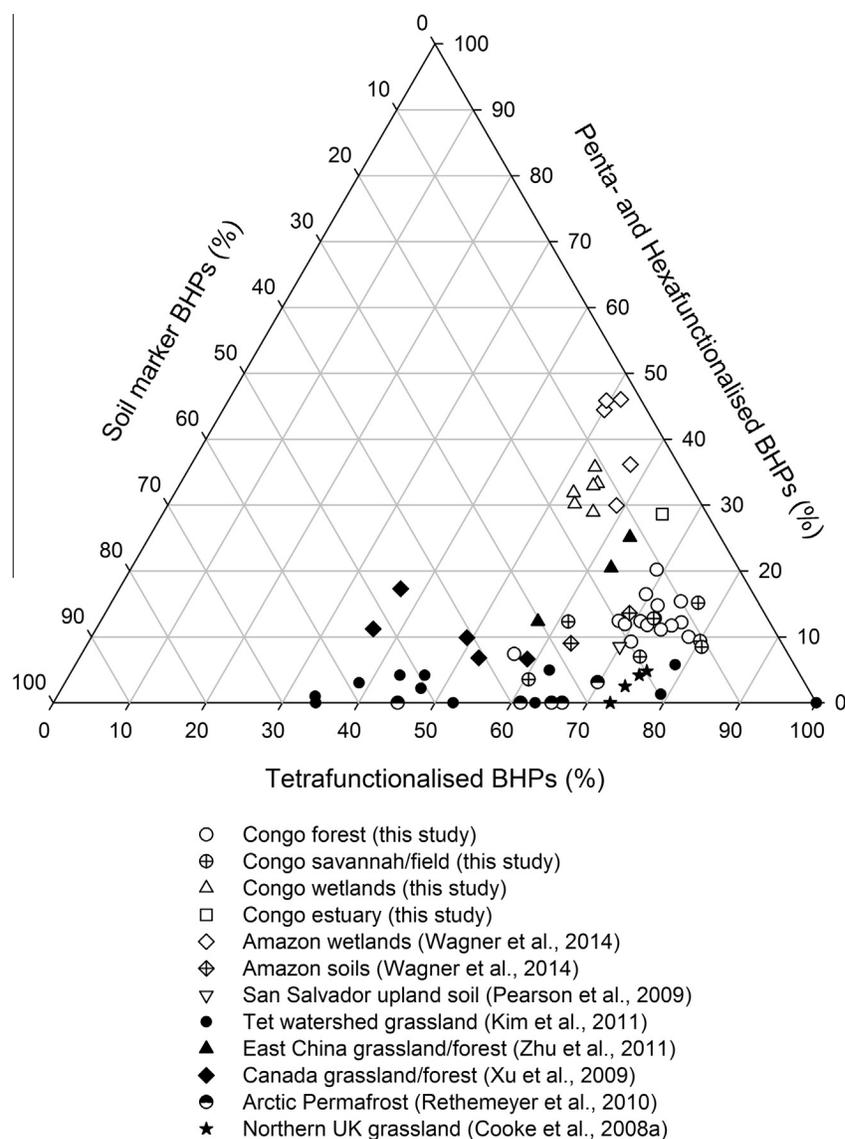
Welander et al., 2009; Schmerk et al., 2011). The pH did not correlate with soil marker BHP concentration ( $\mu\text{g/g}$  TOC;  $r_s = -0.600$ ,  $p = 0.002$ ),  $R_{soil}$  ( $r_s = -0.203$ ,  $p = 0.341$ ) or  $R'_{soil}$  ( $r_s = -0.266$ ,  $p = 0.209$ ). This suggests pH is not a key factor influencing 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 adenomyliopane 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  $R_{soil}$  is 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 6; 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; Rethemeyer et al., 2010; Kim et al., 2011; 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. Studies of surface sediments from river-estuary-shelf/ocean transects have identified a correlation between  $R_{soil}$  and BIT indices (Zhu et al., 2011; Doğrul Selver et al., 2012, 2015). Other studies have not, however, found any correlation between soil marker BHP concentration 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 source materials (soil, peat) is not unexpected; however, it is well established that soil BHP distributions contain variable concentration of the pseudo-marine end member BHT (e.g. Cooke et al., 2008a; Xu et al., 2009; Rethemeyer et al., 2010; Kim et al., 2011), whilst most soils contain little if any crenarchaeol (the marine end member 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 (i) terrestrial end member biomarkers synthesised by microbial organisms living



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

in different environmental niches, for example at different depths in the soil profile (Kim et al., 2011); and (ii) variation in (post-depositional) degradation of terrestrial end member biomarkers due to the differences in compound reactivity (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., 2008b; Handley et al., 2010), this suggests, at least in some settings, that  $R_{\text{soil}}$  could instead be used to describe relative rates of degradation.

#### 4.3. Biomarkers for AMO

Aminopentol (**1f**) is a biomarker for Type I methanotrophs (Rohmer et al., 1984; Neunlist and Rohmer, 1985; 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  $\text{CH}_4$  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 5). The presence of  $\text{CH}_4$  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  $\text{CH}_4$  oxidation marker signatures. Thus, our Congo study is the second to document such a high abundance of  $\text{CH}_4$  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  $\text{CH}_4$  oxidation

**Table 6**  
Summary of CH<sub>4</sub> oxidation markers and soil marker BHPs (% relative total BHPs),  $R_{\text{soil}}$  and  $R'_{\text{soil}}$  plus literature data on surface soil and peat BHP composition.

Location	N	CH <sub>4</sub> oxidation markers (%)		Soil marker BHPs (%)		$R_{\text{soil}}$ ( $R'_{\text{soil}}$ )		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	2.2	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	3.7	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. (2011)
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. (2011)
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. (2008a)

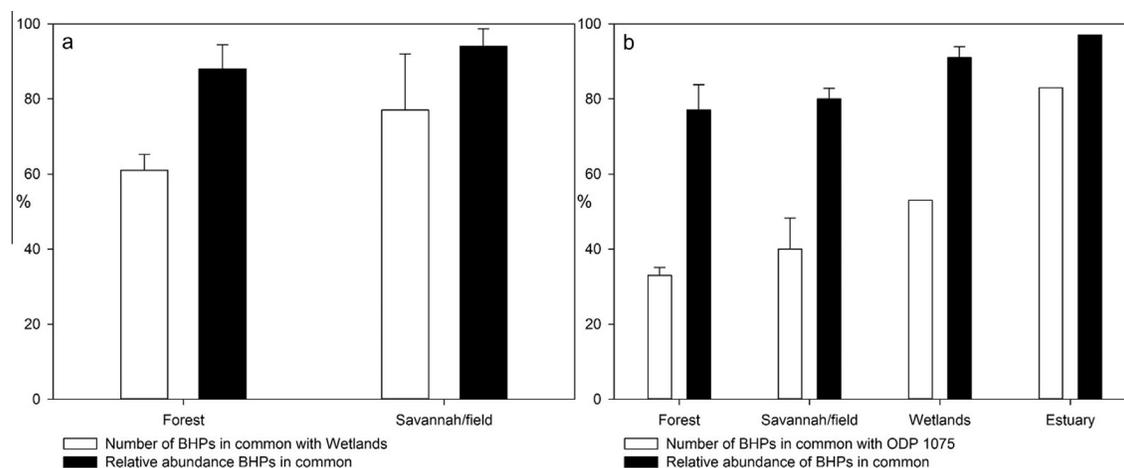
marker, but only a minor compound in the BHP suite overall (Tables 3, 4 and 6). This was unexpected as 2 soils were sampled within an area of methane producing land cover (Fig. 2; swamp forest 11-1; tropical mixed forest 12-1), suggesting AMO should be a significant and readily identifiable from the 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 CH<sub>4</sub> oxidation markers in peatlands, specifically at the oxic-anoxic boundary where AMO is thought to occur. Additionally, Henckel et al. (2001) found that AMO increased 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 CH<sub>4</sub> 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 methanotrophs and at least one Type I methanotroph, *Methylomicrobium 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 drains the second largest basin in the world (ca.  $3.7 \times 10^6$  km<sup>2</sup>). 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 ferralitic soils (Oxisols) that cover large areas of the Congo River basin (Holtvoeth et al., 2005). The OC from the soils analysed here 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  $R_{\text{soil}}$  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 BHP signatures in the wetlands are representative of BHPs from the Congo watershed (Hughes et al., 2011; Spencer et al., 2012). Therefore, a terrestrial  $R_{\text{soil}}$  end member 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 here, the reported  $R_{\text{soil}}$  value of 0.2 (Table 6) may not represent the true mean of the estuary. However, BHT and adenosylhopane concentrations for ODP 1075 have been reported by Handley et al. (2010). Calculation of the  $R_{\text{soil}}$  index for sediments between 10 and 100 Ka ( $n = 27$ ; Appendix II) shows  $R_{\text{soil}}$  index for the estuary is within the range 0.16–0.54 (interglacial 0.16–0.54; glacial 0.21–0.52) for ODP 1075 sediments (Fig. 3). The mean  $R_{\text{soil}}$  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 accord 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 terrigenous (soil) origin.

Furthermore, strong similarities are found between the distribution of BHPs in the soils, wetlands, estuarine and ODP 1075 samples (Fig. 5a and b). A suite of common BHPs occurs in the forest and savannah/field soils, and the wetlands, with more than half of the BHPs in the hinterland soils also present in the wetlands. In addition, the common BHPs 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 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 BHPs in ODP 1075 are also found in the wetlands and soils, and are again a major component of all of the BHP profiles, representing >90% of the total BHPs in the wetland and estuary samples (Fig. 5b). Strong similarities between BHPs in the Congo hinterland and wetland samples and those in ODP 1075 suggest a link between BHP reservoirs. High concentrations of aminotetrol and aminopentol (including aminopentol isomer and unsaturated aminopentol) in ODP 1075 sediments have 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 BHexolcyclitol ether, it is likely that the source of these compounds in the Congo fan is 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.



**Fig. 5.** (a) Mean number of BHPs identified in forest and savannah/field samples in common with wetlands (as % of total number of BHPs; 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 in forest, savannah/field, estuary and wetland samples in common with ODP 1075 (as % of total number of BHPs; 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).

#### 4.5. Trends in global BHP distribution

The data 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) and tetrafunctionalised BHP relative abundance (52–81%) 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 between 34% and 100% (Cooke et al., 2008a; 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., 2008a; Redshaw et al., 2008). High relative abundances 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 of 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% in savannah/field soils) was lower than values for temperate and Arctic surface soils calculated from the available literature data.  $R_{\text{soil}}$  and  $R'_{\text{soil}}$  indices for the soils show a wide 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  $R_{\text{soil}}$  and  $R'_{\text{soil}}$  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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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.orggeochem.2015.09.003>.

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